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Anthranilic acid-based diamides derivatives incorporating aryl-isoxazoline pharmacophore as potential anticancer agents: Design, synthesis and biological evaluation

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1. Introduction

Nowadays, cancer has gradually become the leading diseaserelated cause of deaths of human population, and which has also been seriously threatening the health and life of humans for a long period. Among all the current therapeutic methods, chemotherapy still remains an important option for cancer treatment [1–5]. However, the major hindrance to successful cancer chemotherapy is the development of drug and multidrugresistance, in which cancer cells become simultaneously resistant to different structural types of chemotherapeutic agents [6–9]. Therefore, the discovery and identification of novel highly effective anticancer agents with special characteristics than the currently used is an important endeavor in medicinal chemistry [10–13], and it was necessary to attempt neotype chemical entities as potential chemotherapeutic agents with an alternative mechanism of action.

Recently, diamides derivatives received significant attention for their antitumor properties, especially for those bearing a basic diamide scaffold and pharmacophores [14]. Various diamides derivatives (Fig. 1) have been reported for their wide range of

ABSTRACT

A series of novel anthranilic diamides derivatives containing aryl-isoxazoline moiety were designed and synthesized as a part of our ongoing search for potential anticancer agents. Their structures were confirmed by ¹H NMR, ¹³C NMR and ESI-MS analyses. The preliminary assays showed that some of the compounds displayed moderate to good antitumor activities against human lung cancer (NCI-H460), hepatocellular liver carcinoma (HepG2), gastric cancer (SGC-7901 and BGC-823) and breast epithelial adenocarcinoma (MCF-7) cell lines at µM level, which might be developed as novel lead scaffold for potential anticancer agents.

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pharmacological activities including antitumor (Compds. **1**, **2** and **3**) [7,15,16], antifungal [17], anti-inflammatory activities. In addition, some diamides derivatives are used as Factor Xa inhibitors (Compds. **4** and **5**) [18,19], glycogen phosphorylase inhibitors (Compd. **6**) [20], human sialidase inhibitors (Compd. **7**) [21], and CCK1 receptor antagonists (Compd. **8**) [22] etc. The promising bioactive diversity of this class of diamide compounds urges us to synthesize and biologically evaluate a series of novel structural variants of anthranilic diamides derivatives.

On the other hand, aryl-isoxazoline scaffolds (Fig. 2) have attracted considerable attention for decades and represented an important bioactive heterocycle [23–26]. Recently, numerous studies have demonstrated that this pharmacophore correlates highly with multiple biological activities, and plays an important role in medicinal [27–34] and agrochemical industry [35–38]. In addition, the synthesis and evaluation of new molecular scaffolds based on privileged structures have been identified as a key method in drug discovery process [39,40]. Thus, based on the aforementioned results, we hypothesized that integrating aryl-isoxazoline moiety in anthranilic diamides scaffold (Fig. 2) may lead to novel potential anticancer agents with broad biological activity profiles and improved pharmacokinetic properties.

As part of our medicinal chemistry program aimed at the discovery of potential anticancer agents, we wish to describe herein the molecule design, convenient synthesis, and biological





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Fig. 1. Representative active molecules bearing diamides scaffolds.

evaluation of novel series of anthranilic diamides derivatives containing aryl-isoxazoline pharmacophore. We utilized anthranilic acid scaffold as a key prototype structural unit and planned for the hybridization of the active pharmacophore to the core structure as shown in Fig. 2. Therefore, a series of novel diamides derivatives **6a–v** were designed and synthesized as shown in Scheme 1, and their antitumor activities against various cancer cell lines (HepG2, SGC-7901, BGC-823, NCI-H460, MCF-7) were also evaluated by MTT method.

2. Results and discussion

2.1. Synthesis of anthranilic diamides derivatives

In the present study, a series of novel anthranilic diamides derivatives were constructed by integrating functionalized arylisoxazoline acid 3a-c with substituted 2-aminobenzoic acid 4. The general method for the preparation of anthranilic diamides derivatives containing aryl-isoxazoline moiety 6a-v is outlined in Scheme 1.

The easily available aryl-aldehyde was selected as starting material, which was routinely transferred to the corresponding arylaldoximes **2a–c** by general condensation reaction. The subsequent heterocyclization reaction of compound **2a–c** was treated with α , β unsaturated acid resulting in 4,5-dihydroisoxazolecarboxic acid **3a–c** via classical 1,3-dipolar cycloaddition reaction in the presence of pyridine, and the key heterocyclic acids **3a–c** can be easily separated as colorless crystals with high yields. The derived arylisoxazoline acid **3a–c** can further be conveniently coupled with substituted 2-aminobenzoic acid **4** to form 4*H*-benzo[*d*] [1,3]oxazin-4-one **5a–f** in high yields using methanesulfonyl chloride and pyridine in acetonitrile. The key six-membered lactone intermediates **5a–f** can be directly used to transform to corresponding anthranilic diamides derivatives **6a–v** via routine aminolysis reaction. All the target anthranilic diamides derivatives **6a–v** gave



Fig. 2. Design strategy of anthranilic diamides containing aryl-isoxazoline moiety.

satisfactory chemical analyses, and the chemical structures of the synthesized compounds were summarized in Table 1.

2.2. Pharmacology evaluation

The anthranilic diamides **6a–v** were evaluated for their in vitro cytotoxic effects against HepG2 (hepatocellular liver carcinoma), SGC-7901 (gastric cancer), BGC-823 (gastric cancer), NCI-H460 (lung cancer) and MCF-7 (breast epithelial adenocarcinoma) cell lines by the standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay [41] using **5-FU** (5-Fluorouracil) as a positive control. The results are summarized in Tables 2 and 3. The IC₅₀ value represents the drug concentration required to inhibit cell growth by 50%.

As shown in Table 2, the prepared anthranilic diamides derivatives (6a-v) displayed moderate to good antitumor activities against five human cancer cell lines. In general, the compounds derived from non-substituted 2-aminobenzoic acid (6q-v) exhibited lower activity than the corresponding compounds from substituted 2-aminobenzoic acid (Entry 1–16). The compounds bearing nPr group (6a-c) have relatively high activities than the



Scheme 1. Reagents and conditions: a. $NH_2OH \cdot HCl$, CH_3COONa , MeOH; b. Acrylic acid, NCS, Py, $CHCl_3$; c. $MeSO_2Cl$, Py, MeCN; d. Amine, MeCN, rt for 2–5 h.

 Table 1

 The chemical structure of anthranilic diamides derivatives 6a-v.

Entry	Compd.	Substituents				Yield (%) ^a	Mp (°C)	
	No.	R ¹	\mathbb{R}^2	R ³	\mathbb{R}^4	R ⁵		
1	6a	4-F	Cl	CH_3	Н	CH ₂ CH ₂ CH ₃	64	222-225
2	6b	4-Cl	Cl	CH_3	Н	$CH_2CH_2CH_3$	67	232-234
3	6c	3,4,5-(MeO) ₃	Cl	CH_3	Н	$CH_2CH_2CH_3$	56	190-192
4	6d	4-F	Cl	CH_3	Н	$CH(CH_3)_2$	58	223-225
5	6e	4-Cl	Cl	CH_3	Н	CH(CH ₃) ₂	62	232-235
6	6f	3,4,5-(MeO)3	Cl	CH_3	Н	CH(CH ₃) ₂	48	215-216
7	6g	4-F	Cl	CH_3	Н	Су	56	245-247
8	6h	4-Cl	Cl	CH_3	Н	Су	62	140-142
9	6i	3,4,5-(MeO)3	Cl	CH_3	Н	Су	68	193-194
10	6j	4-F	Cl	CH_3	CH_2CH_3	CH ₂ CH ₃	58	145-147
11	6k	4-Cl	Cl	CH_3	CH_2CH_3	CH ₂ CH ₃	55	153-155
12	61	3,4,5-(MeO)3	Cl	CH_3	CH_2CH_3	CH ₂ CH ₃	63	159-161
13	6m	4-F	Cl	CH_3	Н	CH ₃	54	170-172
14	6n	4-Cl	Cl	CH_3	Н	CH ₃	64	249-251
15	60	3,4,5-(MeO)3	Cl	CH_3	Н	CH ₃	60	187-188
16	6р	4-F	Cl	CH_3	Н	Ph	42	225-226
17	6q	4-F	Н	Н	Н	$CH(CH_3)_2$	55	146-148
18	6r	4-Cl	Н	Н	Н	CH(CH ₃) ₂	63	131-133
19	6s	3,4,5-(MeO)3	Н	Н	Н	CH(CH ₃) ₂	52	198-200
20	6t	4-F	Н	Н	Н	CH ₃	68	195-197
21	6u	4-Cl	Н	Н	Н	CH ₃	70	198-199
22	6v	3,4,5-(MeO) ₃	Н	Н	Н	CH ₃	54	222-224

^a Isolated yields.

compounds containing iPr moiety (**6d**–**f**). Notably, compounds **6i** and **6k** (Entries 9 and 11) exhibited significant inhibitory activity against all tested cell lines with 59.2–76.3 % growth inhibition at 40 μ g/mL concentration. Also, it is interesting to note that compound **6u** (Entry 21) showed cytotoxic selectivity for a special human hepatocellular liver carcinoma cell type, and the inhibition against HepG2 cell was obviously higher than other tested cell lines (Entry 21). The results of preliminary antitumor assay indicated the

Table 2

Cytotoxic activity (% cell growth inhibition) of the compounds 6a-v at 40 $\mu g/mL$ concentration against various human cancer cell lines.

Entry	Compd. No.	Growth-inhibitory properties						
		HepG2 ^a	SGC-7901 ^a	BGC-823 ^a	NCI-H460 ^a	MCF-7 ^a		
1	6a	29.1 ± 4.7	$\textbf{30.5} \pm \textbf{5.3}$	$\textbf{23.5} \pm \textbf{2.2}$	20.0 ± 2.3	$\textbf{33.0}\pm\textbf{0.3}$		
2	6b	$\textbf{16.7} \pm \textbf{4.4}$	14.7 ± 1.7	24.5 ± 4.5	13.6 ± 6.3	$\textbf{22.2} \pm \textbf{4.3}$		
3	6c	21.1 ± 7.5	15.4 ± 6.5	$\textbf{28.0} \pm \textbf{2.7}$	9.5 ± 0.4	29.1 ± 3.4		
4	6d	13.6 ± 1.4	17.2 ± 1.6	0	0	$\textbf{4.8} \pm \textbf{1.4}$		
5	6e	12.3 ± 0.4	24.2 ± 4.6	$\textbf{38.7} \pm \textbf{0.6}$	10.3 ± 2.4	$\textbf{3.9} \pm \textbf{1.6}$		
6	6f	12.9 ± 1.9	0	11.7 ± 4.0	$\textbf{0.5}\pm\textbf{0.2}$	9.1 ± 3.9		
7	6g	10.0 ± 3.4	15.6 ± 2.4	$\textbf{34.3} \pm \textbf{0.4}$	$\textbf{33.3} \pm \textbf{4.0}$	24.8 ± 2.2		
8	6h	15.6 ± 4.1	3.1 ± 0.5	18.3 ± 2.7	15.1 ± 6.0	9.6 ± 2.1		
9	6i	64.0 ± 5.2	60.2 ± 0.7	69.7 ± 3.0	74.0 ± 4.5	$\textbf{62.7} \pm \textbf{4.4}$		
10	6j	18.2 ± 6.2	15.8 ± 1.1	$\textbf{36.4} \pm \textbf{5.5}$	10.0 ± 1.72	14.4 ± 2.1		
11	6k	59.2 ± 5.3	$\textbf{76.3} \pm \textbf{1.4}$	59.6 ± 3.1	$\textbf{63.5} \pm \textbf{5.1}$	62.1 ± 2.6		
12	61	$\textbf{28.6} \pm \textbf{7.0}$	$\textbf{28.3} \pm \textbf{7.4}$	27.8 ± 1.7	0	23.5 ± 1.3		
13	6m	26.7 ± 5.4	12.8 ± 0.1	14.6 ± 3.3	$\textbf{42.9} \pm \textbf{3.8}$	40.8 ± 3.0		
14	6n	18.7 ± 1.1	24.6 ± 6.6	13.2 ± 1.7	0	22.6 ± 2.7		
15	60	$\textbf{30.6} \pm \textbf{3.8}$	$\textbf{27.3} \pm \textbf{3.9}$	34.1 ± 6.9	5.5 ± 3.0	26.1 ± 3.9		
16	6р	5.1 ± 1.3	11.6 ± 2.3	$\textbf{26.4} \pm \textbf{2.1}$	0	17.0 ± 3.0		
17	6q	0	10.3 ± 4.1	21.5 ± 0.7	0	0		
18	6r	5.5 ± 1.3	13.6 ± 2.9	14.6 ± 3.0	0	0		
19	6s	11.7 ± 8.7	0	0	0	0		
20	6t	$\textbf{9.1} \pm \textbf{8.7}$	0	21.4 ± 6.0	0	0		
21	6u	50.6 ± 11.5	$\textbf{7.8} \pm \textbf{2.4}$	13.3 ± 2.5	$\textbf{5.7} \pm \textbf{2.2}$	16.5 ± 6.3		
22	6v	$\textbf{31.4} \pm \textbf{1.2}$	12.0 ± 5.1	20.8 ± 9.1	0	9.1 ± 3.3		
23	5-FU ^b	81.3 ± 2.8	$\textbf{80.8} \pm \textbf{1.4}$	$\textbf{85.7} \pm \textbf{4.4}$	89.7 ± 0.6	$\textbf{80.4} \pm \textbf{3.7}$		

^a Abbreviations: HepG2: human hepatocellular liver carcinoma cell line; SGC-7901: human gastric cancer cell line; BGC-823: human gastric cancer cell line; NCI-H460: human large cell lung cancer cell line; MCF-7: human breast adenocarcinoma cell line.

^b Used as a positive control.

heterocyclic molecule containing trimethoxyphenyl group might be an active scaffold, which further confirmed the compound **6**i should be a potential lead molecule for discovery of *N*-heterocyclic derivatives as potential drugs.

Additionally, for comparison, the selective heterocyclic intermediates 5a-c have also been tested for their in vitro cytotoxic effects. On the basis of the results in Table 3, the intermediate 5aexhibited stronger cytotoxicity than compounds 5b and 5c against all cell lines, and the inhibitory activity against BGC-823 and NCI-H460 for compound 5a is up to 72.1 and 64.6% at tested concentration, respectively. In contrast, compound 5c bearing trimethoxyphenyl group almost lost inhibitory activity at the same concentration. According to the results from Tables 2 and 3, it can be figured out that the diamide products have relatively better activities than the corresponding intermediates, respectively, and the designed anthranilic diamides derivatives containing arylisoxazoline moiety may be used as potential lead compounds for optimization of novel anticancer agents.

On the other hand, since some target compounds displayed relatively higher inhibitory activity against tested cancer cell lines (Table 2), the selective compounds (**6a**, **6c**, **6i**, **6j**, **6k**, **6m**, **6n** and **6o**) were further tested for their inhibitory potential as well as IC₅₀ values. As indicated in Table 4, compound **6i** showed the strongest inhibitory effect against MCF-7 and BGC-823, with an IC₅₀ values of 22.4 and 23.3 μ g mL⁻¹, respectively. While the intermediates **5a** and **5b** were less effective.

Furthermore, the selective dose–response analysis of cell growth inhibition activity for representative compounds **6i**, **6k** has been displayed in Fig. 3, which indicated that the cytotoxic effects on selective cell lines of target compounds showed obvious concentration-dependent manner. Compounds **6i** exhibited potent growth inhibitory activities against BGC-823 and HepG2 cell lines with the IC₅₀ values of 23.3 \pm 4.9 and 34.8 \pm 3.7 $\mu g~mL^{-1}$, respectively.

3. Conclusion

In the present study, we have described the molecular design, synthesis, and biological evaluation of a series of novel anthranilic diamides derivatives containing aryl-isoxazoline pharmacophore. Twenty two novel anthranilic diamides derivatives have been conveniently synthesized, and characterized by ¹H NMR, ¹³C NMR and MS spectra analyses. The preliminary bioassay results indicated that some of the compounds displayed moderate to good antitumor activities against human lung cancer (NCI-H460), hepatocellular liver carcinoma (HepG2), gastric cancer (SGC-7901 and BGC-823) and breast epithelial adenocarcinoma (MCF-7) cell lines, which might be developed as novel lead scaffold for anticancer agents. Further structural optimization and activity profiles about the designed novel diamides derivatives are well ongoing in our laboratory.

4. Materials and methods

4.1. Instrumentation and chemicals

All melting points (m.p.) were obtained with a digital model X-5 apparatus and are uncorrected. ¹H NMR (600 MHz) and ¹³C NMR (100 MHz) spectra were recorded on a Brucker spectrometer with CDCl₃ as the solvent and TMS as the internal standard. Chemical shifts are reported in δ (parts per million) values. Coupling constants ^{*n*}J are reported in Hz. Standard abbreviations indicating multiplicity are used as follows: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quadruplet, m = multiplet and br = broad. Mass spectra were performed on a MicroMass

Table 3

Growth-inhibitory properties for the selective intermediates 5a-c at 40 μ g/mL concentration against various human cancer cell lines.



Entry	Compd. No.	Substituents			Growth-inhibi	Growth-inhibitory properties				
		R ¹	R ²	R ³	HepG2 ^a	SGC-7901 ^a	BGC-823 ^a	NCI-H460 ^a	MCF-7 ^a	
1	5a	4-F	Cl	CH ₃	$\textbf{38.9} \pm \textbf{3.4}$	42.7 ± 4.4	72.1 ± 2.2	64.6 ± 2.0	54.7 ± 6.4	
2	5b	4-Cl	Cl	CH ₃	$\textbf{52.4} \pm \textbf{8.9}$	$\textbf{35.6} \pm \textbf{7.2}$	31.5 ± 3.3	58.1 ± 5.6	$\textbf{37.7} \pm \textbf{5.5}$	
3	5c	3,4,5-(MeO) ₃	Cl	CH ₃	16.5 ± 5.3	0	0	0	12.4 ± 4.6	
4	5-FU ^b	-			81.3 ± 2.8	$\textbf{80.8} \pm \textbf{1.4}$	$\textbf{85.7} \pm \textbf{4.4}$	89.7 ± 0.6	$\textbf{80.4} \pm \textbf{3.7}$	

^a Abbreviations: HepG2: human hepatocellular liver carcinoma cell line; SGC-7901: human gastric cancer cell line; BGC-823: human gastric cancer cell line; NCI-H460: human large cell lung cancer cell line; MCF-7: human breast adenocarcinoma cell line.

^b Used as a positive control.

Quattro $micro^{TM}$ API 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. Anhydrous CH₂Cl₂ and CH₃CN were dried according to standard methods [42]. All other solvents and reagents were analytical reagents and used directly without purification.

4.2. General synthetic procedure for substituted aldoximes 2a-c

To a corresponding substituted benzaldehyde (10 mmol) and hydroxylamine hydrochloride (12 mmol) was added 25 mL of ethanol. The reaction mixture was stirred for 10 min and then sodium acetate (15 mmol) in water (5 mL) was added. The mixture was heated at 45–50 °C during 3–6 h. After completion of the reaction, the mixture was cooled and poured into ice water. The precipitates were filtered off and washed with little ethanol. The product from filtrate was purified by recrystallization. Compound **2a**. (*E*)-4-fluorobenzaldehyde oxime: white powder, yield 94%, m.p. 78–79 °C; Compound **2b**. (*E*)-4-chlorobenzaldehyde oxime: white powder, yield 90%, m.p.99–100.5 °C; Compound **2c**. (*E*)-3,4,5-trimethoxybenzaldehyde oxime: white powder, yield 83%, m.p. 94–95 °C.

Table 4

Selective cytotoxic activity of the compounds against human tumor cells.

Entry	Compd. No.	In vitro cytotoxicity IC_{50}^{a} (µg mL ⁻¹)						
		HepG2 ^b	SGC-7901 ^b	BGC-823 ^b	NCI-H460 ^b	MCF-7 ^b		
1	6a	>100	>100	>100	>100	>100		
2	6c	>100	>100	>100	>100	>100		
3	6i	$\textbf{34.8} \pm \textbf{3.7}$	$\textbf{36.8} \pm \textbf{3.5}$	$\textbf{23.3} \pm \textbf{4.9}$	24.7 ± 6.2	$\textbf{22.4} \pm \textbf{3.5}$		
4	6j	>100	>100	>100	>100	>100		
5	6k	$\textbf{37.7} \pm \textbf{8.8}$	$\textbf{37.4} \pm \textbf{5.9}$	$\textbf{36.5} \pm \textbf{9.7}$	$\textbf{37.5} \pm \textbf{2.4}$	34.5 ± 2.2		
6	6m	>100	>100	>100	$\textbf{50.3} \pm \textbf{2.7}$	>100		
7	6n	>100	>100	>100	>100	>100		
8	60	$\textbf{67.6} \pm \textbf{8.6}$	65.5 ± 4.5	54.1 ± 6.3	>100	>100		
9	5a	$\textbf{53.4} \pm \textbf{6.1}$	53.4 ± 4.0	$\textbf{34.9} \pm \textbf{2.9}$	$\textbf{35.8} \pm \textbf{3.7}$	51.9 ± 7.4		
10	5b	$\textbf{43.6} \pm \textbf{4.9}$	$\textbf{68.8} \pm \textbf{2.1}$	54.4 ± 4.1	41.9 ± 8.4	$\textbf{68.7} \pm \textbf{6.7}$		
11	5c	>100	>100	>100	>100	>100		
12	5-FU ^c	$\textbf{4.8}\pm\textbf{0.7}$	$\textbf{6.3}\pm\textbf{0.8}$	4.5 ± 0.9	2.4 ± 0.5	12.5 ± 2.8		

 $^{\rm a}\,$ IC_{50} - Compound concentration required to inhibit tumor cell proliferation by 50%.

^b Abbreviations: HepG2 – Human hepatocellular liver carcinoma cell line; SGC-7901 – Human gastric cancer cell line; BGC-823 – Human gastric cancer cell line; NCI-H460 – Human large cell lung cancer cell line; MCF-7 – human breast adenocarcinoma cell line.

^c Used as a positive control.

4.3. General synthetic procedure for aryl-isoxazoline acid 3a-c

A solution of the corresponding aryl-aldoxime (2 mmol) in CHCl₃ (2 mL) was added dropwise to an ice-cooled solution of CHCl₃ (8 mL), acrylic acid (2.3 mmol), pyridine (3 mmol) and *N*-chlorosuccinimide (NCS) (0.31 g, 2.3 mmol) under nitrogen for 30 min. The reaction was then stirred at room temperature for several hours and monitored to the completion by thin-layer chromatography. The solution was washed with water and dried with Na₂SO₄. The filtrate was evaporated under a reduced pressure and the residue purified by recrystallization from appropriate solvents. The physico–chemical properties and spectral data for representative compounds are as follows:

4.3.1. Compound 3-(4-fluorophenyl)-4,5-dihydroisoxazole-5carboxylic acid **3a**

This compound was obtained following the above-described method as buff powder, yield 73%, m.p. 148–151 °C; ¹H NMR (600 MHz, CDCl₃): δ = 7.71–7.67 (m, 2H, Ph–H), 7.17–7.11 (m, 2H, Ph–H), 5.28–5.25 (m, 1H, CH–O), 3.74–3.69 (m, 2H, *CH*₂CH).

4.3.2. Compound 3-(4-chlorophenyl)-4,5-dihydroisoxazole-5-carboxylic acid **3b**

This compound was obtained following the above-described method as buff powder, yield 67%, m.p. 164–167 °C; ¹H NMR (600 MHz, CDCl₃): δ = 7.61 (d, *J* = 8.4 Hz, 2H, Ph–H), 7.41 (d, *J* = 8.4 Hz, 2H, Ph–H), 5.26–5.23 (m, 1H, CH–O), 3.71–3.69 (m, 2H, *CH*₂CH).

4.3.3. Compound 3-(3,4,5-trimethoxyphenyl)-4,5-

dihydroisoxazole-5-carboxylic acid 3c

This compound was obtained following the above-described method as off-white powder, yield 70%, m.p. 71–73 °C; ¹H NMR (600 MHz, CDCl₃): δ = 6.90 (s, 2H, Ph–H), 5.23 (t, *J* = 8.7 Hz, 1H, CH–O), 3.91 (s, 9H, CH₃O), 3.71 (d, *J* = 9 Hz, 2H, *CH*₂CH).

4.4. General synthetic procedure for the key intermediates 5a-f

The key intermediates benzoxazinone **5a**–**f** could be conveniently obtained by the coupling of the prepared aryl-isoxazoline acid **3a–c** with substituted anthranilic acids **4**. The general procedures are as follows: To a solution of equivalent aryl-isoxazoline acid **3a–c** (10 mmol) and anthranilic acids **4** (10 mmol) in acetonitrile (50 mL) was added dropwise pyridine (5 mL) and followed by sequential addition of methanesulfonyl chloride (3 mL). The reaction mixture was then stirred at room temperature, and which



Fig. 3. Dose-response analysis of cell growth inhibition activity for representative compounds 6i, 6k and 5-FU (positive control) against BGC-823cells (left) and HepG2 cells (right).

was monitored by thin layer chromatography. After completion of the reaction, the mixture was treated with ice water and formed yellowish solid as crude benzoxazinone **5a**–**f**, which was used for the next reaction without further purification.

4.5. General synthetic procedure for the target compounds 6a-v

The typical process of synthesis of novel anthranilic diamides derivatives containing aryl-isoxazoline 6a-v is shown as following: To a solution of newly prepared benzoxazinone 5a-f (1 mmol) in acetonitrile (10 mL) was added aqueous of methylamine (1 mL) dropwise at room temperature. The reaction mixture was stirred and detected by thin layer chromatography. After completion of the reaction, the solvents were removed by rotary evaporation and the residual solid is purified by silica gel column-chromatography (ethyl acetate/petroleum ether) or recrystallization to give target compounds 6a-v as white solid or crystal. Their physico-chemical properties and the spectra data are as follows:

4.5.1. N-(4-Chloro-2-methyl-6-(propylcarbamoyl)phenyl)-3-(4-fluorophenyl)-4,5-dihydroisoxazole-5-carboxamide **6a**

This compound was obtained following the above method as white floccule, yield 64%, m.p. 222–225 °C; ¹H NMR (600 MHz, CDCl₃) δ 9.25 (s, 1H, NH), 7.71 (t, *J* = 8.4 Hz, 2H, Ph–H), 7.31 (s, 1H, Ph–H), 7.12 (t, *J* = 8.4 Hz, 2H, Ph–H), 5.97 (s, 1H, NH), 5.26 (t, *J* = 7.8 Hz, 1H, CH–O), 3.72 (d, *J* = 7.8 Hz, 2H, *CH*₂–NH), 3.23 (d, *J* = 7.8 Hz, 2H, CH₂–C=N), 2.21 (s, 3H, CH₃–Ph), 1.48 (t, *J* = 7.5 Hz, 2H, *CH*₂CH₃), 0.86 (t, *J* = 7.2 Hz, 3H, CH₂CH₃); ¹³C NMR (100 MHz, CDCl₃): δ 170.09, 166.87, 156.07, 138.09, 134.19, 132.50, 130.94, 129.17, 129.00, 124.95, 116.19, 115.97, 79.24, 41.70, 39.69, 22.71, 18.64, 11.32; ESI-MS: calcd for C₂₁H₂₁ClFN₃O₃ ([M + 1]⁺), 418.1; found, 418.6.

4.5.2. N-(4-Chloro-2-methyl-6-(propylcarbamoyl)phenyl)-3-(4-chlorophenyl)-4,5-dihydroisoxazole-5-carboxamide **6b**

This compound was obtained following the above method as white floccule, yield 67%, m.p. 231.5–234.2 °C; ¹H NMR (600 MHz, CDCl₃) δ 9.26 (s, 1H, NH), 7.65 (d, *J* = 8.4 Hz, 2H, Ph–H), 7.41 (d, *J* = 9.0 Hz, 2H, Ph–H), 7.31 (s, 2H, Ph–H), 5.97 (s, 1H, NH), 5.28–5.25 (m, 1H, CH–O), 3.70 (t, *J* = 5.7 Hz, 2H, *CH*₂–NH), 3.23 (d, *J* = 8.4 Hz, 2H, CH₂–C=N), 2.21 (s, 3H, CH₃–Ph), 1.51–1.47 (m, 2H, *CH*₂CH₃), 0.86 (t, *J* = 7.5 Hz, 3H, CH₂CH₃); ESI-MS: calcd for C₂₁H₂₁Cl₂N₃O₃ ([M + 1]⁺), 434.1; found, 434.5.

4.5.3. N-(4-Chloro-2-methyl-6-(propylcarbamoyl)phenyl)-3-(3,4,5trimethoxyphenyl)-4,5-dihydroisoxazole-5-carboxamide **6c**

This compound was obtained following the above method as white floccule, yield 56%, m.p. 190.2–192.0 °C; ¹H NMR (600 MHz, CDCl₃) δ 9.27 (s, 1H, NH), 7.31 (s, 1H, Ph–H), 7.01 (s, 1H, Ph–H), 6.94 (s, 2H, Ph–H), 5.98 (s, 1H, NH), 5.27–5.24 (m, 1H, CH–O), 3.89 (s,

9H, OCH₃), 3.73 (t, J = 8.4 Hz, 2H, CH_2-NH), 3.27–3.23 (m, 2H, CH₂–C=N), 2.22 (s, 3H, CH₃–Ph), 1.52–1.48 (m, 2H, CH_2CH_3), 0.86 (t, J = 7.2 Hz, 3H, CH₂CH₃); ¹³C NMR (100 MHz, CDCl₃): δ 170.19, 166.87, 156.84, 153.44, 153.39, 140.33, 138.03, 134.83, 134.15, 132.41, 132.29, 130.96, 125.00, 123.79, 123.71, 79.24, 60.96, 56.35, 56.29, 41.71, 39.71, 22.70, 18.66, 11.34; ESI-MS: calcd for C₂₄H₂₈ClN₃O₆ ([M + 1]⁺), 490.2; found, 490.6.

4.5.4. N-(4-Chloro-2-(isopropylcarbamoyl)-6-methylphenyl)-3-(4-fluorophenyl)-4,5-dihydroisoxazole-5-carboxamide **6d**

This compound was obtained following the above method as white floccule, yield 58%, m.p. 223.3–225.1 °C; ¹H NMR (600 MHz, CDCl₃) δ 9.28 (s, 1H, NH), 7.71 (d, *J* = 6.0 Hz, 2H, Ph–H), 7.30 (s, 1H, Ph–H), 7.24 (s, 1H, Ph–H), 7.16 (t, *J* = 8.4 Hz, 2H, Ph–H), 5.77 (d, *J* = 9.0 Hz, 1H, NH), 5.27–5.24 (m, 1H, CH–O), 4.10–4.08 (m, 1H, CH–NH), 3.73 (d, *J* = 6.0 Hz, 2H, CH₂–C=N), 2.20 (s, 3H, CH₃–Ph), 1.19 (d, *J* = 6.6 Hz, 3H, CH₃), 1.07 (d, *J* = 6.0 Hz, 3H, CH₃); ESI-MS: calcd for C₂₁H₂₁ClFN₃O₃ ([M + 1]⁺), 418.1; found, 418.5.

4.5.5. N-(4-Chloro-2-(isopropylcarbamoyl)-6-methylphenyl)-3-(4chlorophenyl)-4,5-dihydroisoxazole-5-carboxamide **6**e

This compound was obtained following the above method as white floccule, yield 62%, m.p. 232–235 °C; ¹H NMR (600 MHz, CDCl₃) δ 9.28 (s, 1H, NH), 7.64 (d, *J* = 8.4 Hz, 2H, Ph–H), 7.40 (d, *J* = 9.0 Hz, 2H, Ph–H), 7.30 (s, 1H, Ph–H), 7.24 (s, 1H, Ph–H), 5.77 (d, *J* = 7.8 Hz, 1H, NH), 5.28–5.25 (m, 1H, CH–O), 4.10–4.08 (m, 1H, CH–NH), 3.72 (d, *J* = 7.8 Hz, 2H, CH₂–C=N), 2.20 (s, 3H, CH₃–Ph), 1.19 (d, *J* = 7.2 Hz, 3H, CH₃), 1.08 (d, *J* = 6.6 Hz, 3H, CH₃); ESI-MS: calcd for C₂₁H₂₁Cl₂N₃O₃ ([M + 1]⁺), 434.1; found, 434.5.

4.5.6. N-(4-Chloro-2-(isopropylcarbamoyl)-6-methylphenyl)-3-

(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole-5-carboxamide **6f** This compound was obtained following the above method as white floccule, yield 48%, m.p. 215–216 °C; ¹H NMR (600 MHz, CDCl₃) δ 9.32 (s, 1H, NH), 7.31 (s, 1H, Ph–H), 7.25 (d, 1H, Ph–H), 6.93 (s, 2H, Ph–H), 5.79 (d, *J* = 7.8 Hz, 1H, NH), 5.27–5.24 (m, 1H, CH–O),

4.13–4.09 (m, 1H, *CH*–NH), 3.89 (s, 9H, OCH₃), 3.77–3.70 (m, 2H, CH₂–C=N), 2.21 (s, 3H, CH₃–Ph), 1.19 (d, *J* = 6.6 Hz, 3H, CH₃), 1.09 (d, *J* = 6.0 Hz, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃): δ 170.12, 166.14, 156.85, 153.36, 140.32, 138.01, 134.31, 132.32, 132.26, 130.91, 124.96, 123.70, 104.46, 79.26, 60.96, 56.28, 42.04, 39.63, 22.49, 22.39, 18.65; ESI-MS: calcd for C₂₄H₂₈ClN₃O₆ ([M + 1]⁺), 490.2; found, 490.6.

4.5.7. N-(4-Chloro-2-(cyclohexylcarbamoyl)-6-methylphenyl)-3-(4-fluorophenyl)-4,5-dihydroisoxazole-5-carboxamide **6g**

This compound was obtained following the above method as white floccule, yield 56%, m.p. 245.2–247.0 °C; ¹H NMR (600 MHz, CDCl₃) δ 9.28 (s, 1H, NH), 7.72–7.69 (m, 2H, Ph–H), 7.30 (s, 1H, Ph–H), 7.24 (s, 1H, Ph–H), 7.12 (t, *J* = 8.4 Hz, 2H, Ph–H), 5.81 (d, *J* = 8.4 Hz, 1H, NH), 5.27–5.25 (m, 1H, CH–O), 3.78–3.70 (m, 3H,

CH–NH and CH₂–C=N), 2.20 (s, 3H, CH₃–Ph), 1.95–1.54 (m, 10H, (CH₂)₅); ESI-MS: calcd for C₂₄H₂₅CIFN₃O₃ ([M + 1]⁺), 458.2; found, 458.6.

4.5.8. N-(4-Chloro-2-(cyclohexylcarbamoyl)-6-methylphenyl)-3-(4-chlorophenyl)-4,5-dihydroisoxazole-5-carboxamide **6h**

This compound was obtained following the above method as white floccule, yield 62%, m.p. 140.2–141.5 °C; ¹H NMR (600 MHz, CDCl₃) δ 9.21 (s, 1H, NH), 7.93 (s, 1H, Ph–H), 7.64 (d, *J* = 8.4 Hz, 2H, Ph–H), 7.40 (d, *J* = 9.0 Hz, 2H, Ph–H), 7.31 (s, 1H, Ph–H), 6.06 (bs, 1H, NH), 5.01 (s, 1H, CH–O), 3.86 (m, 1H, N–CH), 3.22 (d, 2H, CH₂–C=N), 2.21 (s, 3H, CH₃–Ph), 1.94–1.59 (m, 10H, (CH₂)₅); ESI-MS: calcd for C₂₄H₂₅Cl₂N₃O₃ ([M + 1]⁺), 474.1; found, 474.6.

4.5.9. N-(4-Chloro-2-(cyclohexylcarbamoyl)-6-methylphenyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole-5-carboxamide **6i**

This compound was obtained following the above method as white floccule, yield 68%, m.p. 193.3–193.5 °C; ¹H NMR (600 MHz, CDCl₃) δ 9.27 (s, 1H, NH), 7.30 (s, 1H, Ph–H), 7.25 (s, 1H, Ph–H), 6.93 (s, 2H, Ph–H), 5.84 (d, *J* = 7.8 Hz, 1H, NH), 5.27–5.24 (m, 1H, CH–O), 3.89 (s, 9H, OCH₃), 3.78–3.67 (m, 3H, N–CH and CH₂–C=N), 2.21 (s, 3H, CH₃–Ph), 1.98–1.52 (m, 10H, (CH₂)₅); ESI-MS: calcd for C₂₇H₃₂ClN₃O₆ ([M + 1]⁺), 530.2; found, 530.9.

4.5.10. N-(4-Chloro-2-(diethylcarbamoyl)-6-methylphenyl)-3-(4-fluorophenyl)-4,5-dihydroisoxazole-5-carboxamide **6**j

This compound was obtained following the above method as light yellow floccule, yield 58%, m.p. 144.9–147.2 °C; ¹H NMR (600 MHz, CDCl₃) δ 8.61 (s, 1H, NH), 7.69 (s, 2H, Ph–H), 7.25 (s, 1H, Ph–H), 7.14–7.11 (m, 2H, Ph–H), 7.07 (s, 1H, Ph–H), 5.23 (s, 1H, CH–O), 3.69 (d, *J* = 7.8 Hz, 2H, N–CH₂), 3.23–3.15 (m, 4H, N–CH₂ and CH₂–C=N), 2.18 (s, 3H, CH₃–Ph), 1.10 (s, 3H, CH₃), 0.98 (s, 3H, CH₃); ESI-MS: calcd for C₂₂H₂₃ClFN₃O₃ ([M + 1]⁺), 432.1; found, 432.7.

4.5.11. N-(4-Chloro-2-(diethylcarbamoyl)-6-methylphenyl)-3-(4chlorophenyl)-4,5-dihydroisoxazole-5-carboxamide **6k**

This compound was obtained following the above method as white floccule, yield 55%, m.p. 152.7–155.2 °C; ¹H NMR (600 MHz, CDCl₃) δ 8.55 (s, 1H, NH), 7.63 (d, *J* = 7.2 Hz, 2H, Ph–H), 7.41 (d, *J* = 8.4 Hz, 2H, Ph–H), 7.25 (s, 1H, Ph–H), 7.07 (s, 1H, Ph–H), 5.23 (s, 1H, CH–O), 3.68 (d, *J* = 7.2 Hz, 2H, N–CH₂), 3.25–3.16 (m, 4H, N–CH₂ and CH₂–C=N), 2.19 (s, 3H, CH₃-Ph), 1.10 (s, 3H, CH₃), 0.98 (s, 3H, CH₃); ESI-MS: calcd for C₂₂H₂₃Cl₂N₃O₃ ([M + 1]⁺), 448.1; found, 448.6.

4.5.12. N-(4-Chloro-2-(diethylcarbamoyl)-6-methylphenyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole-5-carboxamide **6**

This compound was obtained following the above method as white floccule, yield 63%, m.p. 159.2–160.7 °C; ¹H NMR (600 MHz, CDCl₃) δ 8.58 (s, 1H, NH), 7.26 (s, 1H, Ph–H), 7.08 (s, 1H, Ph–H), 6.91 (s, 2H, Ph–H), 5.22 (s, 1H, CH–O), 3.89 (s, 9H, OCH₃), 3.70 (d, J = 6.6 Hz, 2H, N–CH₂), 3.25–3.15 (m, 4H, N–CH₂ and CH₂–C=N), 2.20 (s, 3H, CH₃–Ph), 1.13 (s, 3H, CH₃), 0.99 (s, 3H, CH₃); ESI-MS: calcd for C₂₅H₃₀ClN₃O₆ ([M + 1]⁺), 504.2; found, 504.8.

4.5.13. N-(4-Chloro-2-methyl-6-(methylcarbamoyl)phenyl)-3-(4-fluorophenyl)-4,5-dihydroisoxazole-5-carboxamide **6m**

This compound was obtained following the above method as white floccule, yield 54%, m.p. 169.5–172.0 °C; ¹H NMR (600 MHz, CDCl₃) δ 9.27 (s, 1H, NH), 8.09 (s, 1H, Ph–H), 7.78 (s, 2H, Ph–H), 7.72 (s, 1H, NH), 7.52 (s, 1H, Ph–H), 7.16–7.12 (m, 2H, Ph–H), 5.91–5.88 (m, 1H, CH–O), 4.80–4.75 (m, 1H, CH₂–C=N), 3.84 (s, 3H, N–CH₃), 3.58–3.53 (m, 1H, CH₂–C=N), 2.49 (s, 3H, CH₃–Ph); ESI-MS: calcd for C₁₉H₁₇ClFN₃O₃ ([M + 1]⁺), 390.1; found, 390.6.

4.5.14. N-(4-Chloro-2-methyl-6-(methylcarbamoyl)phenyl)-3-(4chlorophenyl)-4,5-dihydroisoxazole-5-carboxamide **6n**

This compound was obtained following the above method as white floccule, yield 64%, m.p. 249.1–250.6 °C; ¹H NMR (600 MHz, CDCl₃) δ 9.31 (s, 1H, NH), 8.09 (s, 1H, Ph–H), 7.71–7.41 (m, 6H, Ph–H and NH), 5.90 (s, 1H, CH–O), 4.78–4.75 (m, 1H, CH₂–C=N), 3.84 (s, 3H, N–CH₃), 3.58–3.55 (m, 1H, CH₂–C=N), 2.19 (s, 3H, CH₃–Ph); ESI-MS: calcd for C₁₉H₁₇Cl₂N₃O₃ ([M + 1]⁺), 406.1; found, 406.8.

4.5.15. N-(4-Chloro-2-methyl-6-(methylcarbamoyl)phenyl)-3-

 $\begin{array}{l} (3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole-5-carboxamide \ {\bf 60} \\ \mbox{This compound was obtained following the above method as white floccule, yield 60%, m.p. 186.5–188.0 °C; ¹H NMR (600 MHz, CDCl₃) <math display="inline">\delta$ 9.38 (s, 1H, NH), 7.29–7.25 (m, 3H, Ph–H and NH), 7.01 (s, 2H, Ph–H), 6.22 (s, 1H, CH–O), 5.28–5.25 (m, 1H, CH₂–C=N), 3.92–3.83 (m, 10H, CH₃O and CH₂–C=N), 2.83 (s, 3H, CH₃–N), 2.20 (s, 3H, CH₃–Ph); ¹³C NMR (100 MHz, CDCl₃): δ 170.19, 167.40, 156.87, 153.38, 140.33, 137.87, 133.57, 132.47, 132.21, 131.04, 125.11, 123.68, 104.41, 79.24, 60.97, 56.34, 56.29, 39.74, 26.67, 18.66; ESI-MS: calcd for C₂₂H₂₄ClN₃O₆ ([M + 1]⁺), 462.1; found, 462.7.

4.5.16. N-(4-Chloro-2-methyl-6-(phenylcarbamoyl)phenyl)-3-(4-fluorophenyl)-4,5-dihydroisoxazole-5-carboxamide **6**p

This compound was obtained following the above method as white floccule, yield 42%, m.p. 224.7–226.2 °C; ¹H NMR (600 MHz, CDCl₃) δ 8.83 (s, 1H, NH), 7.90 (s, 1H, NH), 7.46–7.35 (m, 7H, Ph–H), 7.24–7.02 (m, 4H, Ph–H), 5.23 (s, 1H, CH–O), 3.62–3.55 (m, 2H, CH₂–C=N), 2.25 (s, 3H, CH₃–Ph); ESI-MS: calcd for C₂₄H₂₅CIFN₃O₃ ([M + 1]⁺), 458.2; found, 458.7.

4.5.17. 3-(4-Fluorophenyl)-N-(2-(isopropylcarbamoyl)phenyl)-4,5dihydroisoxazole-5-carboxamide **6q**

This compound was obtained following the above method as white floccule, yield 55%, m.p. 146.0–148.2 °C; ¹H NMR (600 MHz, CDCl₃) δ 12.05 (s, 1H, NH), 8.59 (d, *J* = 8.4 Hz, 1H, Ph–H), 8.26 (d, *J* = 7.8 Hz, 1H, Ph–H), 7.65–7.36 (m, 4H, Ph–H), 5.93 (bs, 1H, NH), 5.30–5.27 (m, 1H, CH–O), 4.37–4.34 (m, 1H, *CH*–NH), 3.77–3.67 (m, 2H, CH₂–C=N), 1.27–1.12 (m, 6H, CH₃); ESI-MS: calcd for C₂₀H₂₀FN₃O₃ ([M + 1]⁺), 370.1; found, 370.6.

4.5.18. 3-(4-Chlorophenyl)-N-(2-(isopropylcarbamoyl)phenyl)-4,5dihydroisoxazole-5-carboxamide **6r**

This compound was obtained following the above method as white floccule, yield 63%, m.p. 131.4–132.6 °C; ¹H NMR (600 MHz, CDCl₃) δ 12.05 (s, 1H, NH), 8.59 (d, *J* = 8.4 Hz, 1H, Ph–H), 7.64 (d, *J* = 8.4 Hz, 2H, Ph–H), 7.47–7.36 (m, 4H, Ph–H), 7.12–7.09 (m, 1H, Ph–H), 5.94 (bs, 1H, NH), 5.29–5.27 (m, 1H, CH–O), 4.37–4.34 (m, 1H, *CH*–NH), 3.78–3.67 (m, 2H, CH₂–C=N), 1.33–1.24 (m, 6H, CH₃); ¹³C NMR (100 MHz, CDCl₃): δ 169.82, 167.80, 159.63, 156.15, 155.91, 133.54, 132.84, 132.20, 129.26, 129.02, 128.33, 128.20, 123.76, 79.59, 41.95, 39.49, 22.72; ESI-MS: calcd for C₂₀H₂₀ClN₃O₃ ([M + 1]⁺), 386.1; found, 386.5.

4.5.19. N-(2-(Isopropylcarbamoyl)phenyl)-3-(3,4,5-

trimethoxyphenyl)-4,5-dihydroisoxazole-5-carboxamide 6s

This compound was obtained following the above method as white floccule, yield 52%, m.p. 198.2–199.8 °C; ¹H NMR (600 MHz, CDCl₃) δ 12.08 (s, 1H, NH), 8.59 (s, 1H, Ph–H), 7.45 (s, 2H, Ph–H), 7.12 (s, 1H, Ph–H), 6.95 (s, 2H, Ph–H), 5.93 (bs, 1H, NH), 5.29–5.26 (m, 1H, CH–O), 4.36–4.34 (m, 1H, *CH*–NH), 3.87 (s, 9H, OCH₃), 3.80–3.71 (m, 2H, CH₂–C=N), 1.27 (d, *J* = 6.0 Hz, 3H, CH₃), 1.24 (d, *J* = 6.0 Hz, 3H, CH₃); ESI-MS: calcd for C₂₃H₂₇N₃O₆ ([M + 1]⁺), 442.2; found, 442.6.

4.5.20. 3-(4-Fluorophenyl)-N-(2-(methylcarbamoyl)phenyl)-4,5dihydroisoxazole-5-carboxamide **6t**

This compound was obtained following the above method as white floccule, yield 68%, m.p. 195.2–196.8 °C; ¹H NMR (600 MHz, CDCl₃) δ 12.43 (s, 1H, NH), 8.71 (s, 1H, Ph–H), 8.14 (s, 1H, Ph–H), 7.66–7.57 (m, 3H, Ph–H), 7.16–7.06 (m, 3H, Ph–H), 5.30–5.20 (m, 1H, CH–O), 3.81–3.72 (m, 2H, CH₂–C=N), 3.04 (s, 3H, NCH₃); ESI-MS: calcd for C₁₈H₁₆FN₃O₃ ([M + 1]⁺), 342.1; found, 342.7.

4.5.21. 3-(4-Chlorophenyl)-N-(2-(methylcarbamoyl)phenyl)-4,5dihydroisoxazole-5-carboxamide **6u**

This compound was obtained following the above method as light yellow crystal, yield 70%, m.p. 197.8–199.0 °C; ¹H NMR (600 MHz, CDCl₃) δ 12.20 (s, 1H, NH), 8.62 (d, *J* = 7.8 Hz, 1H, Ph–H), 7.65 (d, *J* = 8.4 Hz, 2H, Ph–H), 7.48 (d, *J* = 7.8 Hz, 2H, Ph–H), 7.47 (d, *J* = 7.2 Hz, 2H, Ph–H), 7.11 (t, *J* = 7.2 Hz, 1H, Ph–H), 6.27 (s, 1H, NH), 5.30–5.27 (m, 1H, CH–O), 3.76–3.70 (m, 2H, CH₂–C=N), 3.04 (s, 3H, NCH₃); ESI-MS: calcd for C₁₈H₁₆ClN₃O₃ ([M + 1]⁺), 358.1; found, 358.6.

4.5.22. N-(2-(Methylcarbamoyl)phenyl)-3-(3,4,5trimethoxyphenyl)-4.5-dihydroisoxazole-5-carboxamide **6v**

This compound was obtained following the above method as white floccule, yield 54%, m.p. 221.6–224.1 °C; ¹H NMR (600 MHz, CDCl₃) δ 12.23 (s, 1H, NH), 8.63 (d, *J* = 7.8 Hz, 1H, Ph–H), 7.47 (t, *J* = 7.8 Hz, 2H, Ph–H), 7.11 (t, *J* = 7.2 Hz, 1H, Ph–H), 6.95 (s, 2H, Ph–H), 6.28 (s, 1H, NH), 5.28 (d, *J* = 7.2 Hz, 1H, CH–O), 3.93–3.72 (m, 11H, CH₂–C=N and OCH₃), 3.04 (s, 3H, NCH₃); ¹³C NMR (100 MHz, CDCl₃): δ 170.15, 168.91, 156.66, 153.41, 153.30, 140.13, 138.30, 132.34, 127.61, 126.56, 123.95, 123.54, 121.34, 104.45, 79.48, 60.95, 56.34, 56.28, 39.77, 26.96; ESI-MS: calcd for C₂₁H₂₃N₃O₆ ([M + 1]⁺), 414.2; found, 414.8.

5. In vitro cytotoxicity assays

The in vitro cytotoxicity of the synthesized compounds against different human cancer cell lines was measured with the 3-(4,5dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay [41]. Briefly, HepG2 (hepatocellular liver carcinoma), SGC-7901 (gastric cancer), BGC-823 (gastric cancer), NCI-H460 (lung cancer) and MCF-7 (breast epithelial adenocarcinoma) cells were seeded at 2×10^4 cells per well in 96-well plates and grown to subconfluence. After removal of the growth medium, six serial dilutions of each tested compound in 200 µL test medium were added. Plates were incubated at 37 °C in a humidified atmosphere containing 5% CO₂. After 72 h of exposure, the culture medium was removed and 30 µL of the MTT solution (5 mg/mL in PBS) was added to each well. The plate was further incubated for 4 h to allow MTT formazan formation. To dissolve the resulting MTT formazan, 50 uL of DMSO was added to each well, followed by thorough mixing with a microplate shaker. Absorbance at 570 nm was measured on a microplate reader (Thermo Scientific, MK3). All data were analyzed with SPSS software, and the 50% inhibitory concentrations (IC₅₀) of each compound for the different cell lines were determined. Assays were performed in triplicate on three independent experiments.

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Appendix A. Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.ejmech.2012.06.001.

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