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Biological Evaluation of 4-Aryl-1,4-dihydropyridines as VEGFR-2 Kinase Inhibitors[1]

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Abstract—Vascular endothelial growth factor-2 receptor (VEGFR-2) kinase is a promising target for the development of novel anticancer drugs. Molecular docking modeling was performed on a series of 4-aryl-1,4-dihydropyridines derivatives to evaluate the structural basis for VEGFR-2 inhibitory activity. Some 4-aryl-1,4-dihydropyridines were synthesized in the reaction of aromatic aldehydes and ethyl propiolate with anilines in acetic acid. The biological activities were evaluated against the cells A549, A431 and Hep-G2. The results indicated that 4-aryl-1,4-dihydropyridines could be the promising potential VEGFR-2 inhibitors.

Keywords: VEGFR-2 inhibitors, 4-aryl-1,4-dihydropyridines, molecular docking, biological activity

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INTRODUCTION

Vascular endothelial growth factor-2 receptor (VEGFR-2) is an important member of the Receptor Tyrosine Kinases (RTKs) family and widely studied in the regulating tumor angiogenesis [1, 2]. VEGFR-2 signaling promotes several endothelial responses such as cell proliferation, migration and survival that are required for the formation of new blood vessels [3]. Activation of VEGFR-2 is correlated with poor prognosis and metastasis in the majority of solid tumor patients. Inhibition of VEGFR-2 signaling blocks the new blood vessels formation in growing tumors and results in stasis or suppression of tumor growth [4, 5]. The development of small-molecule VEGFR-2 kinase inhibitors for anticancer therapy has initiated active pharmaceutical research. At present, many structurally diverse VEGFR-2 inhibitors have been approved for therapeutic treatment, including Vatalanib, BMS-794833 and Sorafenib Tosylate (Fig. 1) [6–8].

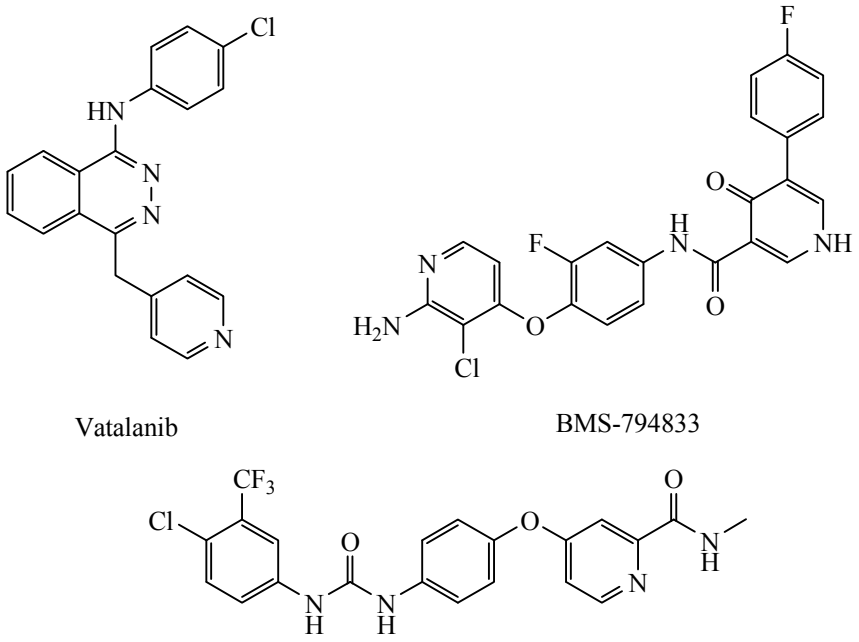
1,4-Dihydropyridines make a class of calcium antagonists that oppose the entry of calcium into cells and reduce the influx of calcium ions through the slow calcium channels in the cells [9]. However, it was considered that the 1,4-dihydropyridines have an influence on tumor cells [10, 11]. Based on the molecular modelling of the interactions between the known inhibitors and VEGFR-2, there was presented a typical pharmacophore model for the inhibitors with central nitrogen heterocycles occupying the important region. Such nitrogen heterocycles constitute an important scaffold for VEGFR-2 inhibitors and provide some information about VEGFR-2 kinase inhibitors [12–14]. Herein, we describe an on-going effort to develop small-molecule VEGFR-2 kinase inhibitors based on the 1,4-dihydropyridines scaffold. Molecular docking modeling was performed for elucidating the benefit of 4-aryl-1,4-dihydropyridines for VEGFR-2 inhibition. A series of 4-aryl-1,4-dihydropyridines was synthesized by the reaction of aromatic aldehydes and ethyl propiolate with anilines in acetic acid and evaluated for inhibitory activity against A549 (small cell lung cancer) cells in vitro using a MTT assay.

RESULTS AND DISCUSSION

Molecular docking. On the basis of the crystal structure of VEGFR-2 complexed with a Sorafenib tosylate inhibitor (PDB code: 4ASD), AutoDock 4.0 (Scripps Research Institute, La Jolla, CA, USA) was used to simulate 4-aryl-1,4-dihydropyridines (**1**) [15]. The docking results (Table 1) and are compared with those of the known inhibitors **1–3**.

According to data presented in Table 1, compounds **1** exhibited higher binding capacity with VEGFR-2 than the known inhibitors. Free energy ΔG and

[1] The text was submitted by the authors in English.



Vatalanib BMS-794833

Sorafenib Tosylate

Fig. 1. Representative VEGFR-2 kinase inhibitors.

inhibition constant values K_i of 1 were between –7 and –5 kcal/mol and between 4 and 150 μM, respectively. The binding capacities were similar to those obtained from the docking of the known inhibitors (ΔG = –6.41, –6.53, and 6.42 kcal/mol, K_i = 20.12, 16.44, and 19.62 μM, respectively). The compound **1m'** (R_1 = 2,3,4-triOCH_3, R_2 = Bn) exhibited higher binding capacity with VEGFR-2 (ΔG = 7.34 kcal/mol, K_i = 4.19 μM). The substituents R_1 and R_2 probably could influence upon the binding capacity. In cases of R_1 = OCH_3 or NO_2 and R_2 = Bz the binding capacity increased significantly.

The sites and modes of interactions between ligands and VEGFR-2 were considered. For the compounds **1w** and **1m'** and the known inhibitors a similar binding site to VEGFR-2 was detected and the compounds **1w** and **1m'** demonstrated high overlap ratios with Vatalanib and BMS-794833 in the binding site of the VEGFR-2 kinase (Fig. 2), which was consistent with the biological activities data. For the known inhibitors, the hydrogen bonds with the enzyme backbone were detected. In the inhibitor BMS-794833 the NH_2 group formed two H-bonds with the ARG840 carboxyl group. The C=O group formed the third H-bond with the backbone N–H of PHE1045 (Fig. 3a). In the inhibitor Sorafenib Tosylate the NH group formed the H-bond with the ASP1026 carboxyl group (Fig. 3b).

The compounds **1w** and **1m'** were also involved in hydrogen bonding with the enzyme backbone. The –$COOCH_3$ group formed two H-bonds with the CYS1043 sulfhydryl and the –OCH_3 groups in meta- and para-positions formed other three H-bonds with the backbone N–H of ASN921 (Figs. 3d, 3f). For the compound **1w**, the –OCH_3 groups in meta-position formed the H-bond with the CYS1043 sulfhydryl group (Figs. 3c, 3e). The –OCH_3 groups could make the molecule ligand integrated closely with amino acid of the receptor by the H-bond. However, **1w** was involved in the π–π interaction between the benzene ring and the amino acid PHE916. The protein cavity could be totally occupied by the bulky Ph group of **1w** which strengthened stability of the complex constituted by the ligand and receptor ultimately. The above could probably explain why **1w** and **1m'** exhibited the higher binding capacity for H-bonds with the enzyme backbone and the benzyl transforming the core of **1** into an acceptable conformation within the active site of VEGFR-2.

Synthesis of 4-aryl-1,4-dihydropyridines. According to the results of molecular docking, some of 4-aryl-1,4-dihydropyridines were selected for the study. Based on previously reported data [16, 17], 4-aryl-1,4-dihydropyridines **1** were synthesized in the reaction of aromatic aldehydes and ethyl propiolate with anilines

Table 1. Molecular docking data for **1** and the known inhibitors with VEGFR-2

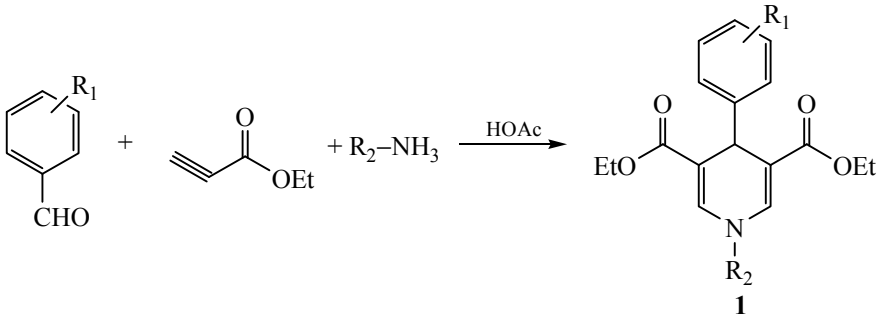
Comp. no.	R_1	R_2	ΔG^a	K_i^b	Comp. no.	R_1	R_2	ΔG^a	K_i^b
1a	H	H	5.13	172.92	**1w**	H	4-CH_3-Ph	5.55	85.31
1b	4-CH_3	H	5.18	158.38	**1x**	H	4-Cl-Ph	5.33	125.02
1c	4-OH	H	5.66	70.51	**1y**	H	4-F-Ph	5.24	143.70
1d	4-Cl	H	4.70	359.93	**1z**	H	4-NO_2-Ph	6.59	14.70
1e	4-F	H	5.49	94.51	**1a'**	H	4-OCH_3-Ph	6.13	32.28
1f	4-NO_2	H	6.10	33.70	**1b'**	H	2,4-diOCH_3-Ph	5.63	60.43
1g	4-CF_3	H	4.86	274.68	**1c'**	H	3,4,5-triCH_3-Ph	5.35	119.82
1h	4-OCH_3	H	5.25	141.15	**1d'**	H	Bn	6.69	12.44
1i	2,4-diOCH_3	H	4.38	613.15	**1e'**	4-CH_3	Bn	6.92	8.46
1j	2,3,4-triOCH_3	H	4.98	224.01	**1f'**	4-OH	Bn	6.75	11.29
1k	2,4,5-triOCH_3	H	5.06	196.03	**1g'**	4-Cl	Bn	6.99	7.57
1l	3,4,5-triOCH_3	H	4.80	303.73	**1h'**	4-F	Bn	6.60	14.40
1m	H	Ph	5.45	100.49	**1i'**	4-NO_2	Bn	7.05	6.74
1n	4-CH_3	Ph	5.85	51.44	**1j'**	4-CF_3	Bn	6.26	25.81
1o	4-OH	Ph	5.24	143.70	**1k'**	4-OCH_3	Bn	6.64	13.49
1p	4-Cl	Ph	5.37	116.36	**1l'**	2,4-diOCH_3	Bn	6.68	12.68
1q	4-F	Ph	5.67	69.77	**1m'**	2,3,4-triOCH_3	Bn	7.34	4.19
1r	4-NO_2	Ph	6.26	25.81	**1n'**	2,4,5-triOCH_3	Bn	7.43	3.55
1s	4-OCH_3	Ph	6.53	16.44	**1o'**	3,4,5-triOCH_3	Bn	7.29	4.53
1t	2,4-diOCH_3	Ph	5.40	109.91	Vatalanib	–	–	6.41	20.12
1u	2,4,5-triOCH_3	Ph	5.68	68.34	BMS-794833	–	–	6.53	16.44
1v	3,4,5-triOCH_3	Ph	6.75	11.29	Sorafenib Tosylate	–	–	6.42	19.62

[a] Binding free energy (kcal/mol). [b] Inhibition constant (μM).

in acetic acid (Scheme 1). The structures of products 1 were confirmed by ^{1}H NMR.

Biological evaluation. The preliminary biological activity of 4-aryl-1,4-dihydropyridines on VEGFR-2 kinase was evaluated against A549 (lung carcinoma) cells, A431 (epidermoid carcinoma) cells and Hep-G2 (hepatocellular carcinoma) cells using a MTT assay [18, 19]. Taxol was used as a reference drug. The biological activity data of 4-aryl-1,4-dihydropyridines

Scheme 1. Synthesis of 4-aryl-1,4-dihydropyridines **1.**



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R_1 = H, 4-CH_3, 4-OH, 4-Cl, 4-F, 4-NO_2, 4-CF_3, 4-OCH_3, 2,4-diOCH_3, 2,3,4-triOCH_3, 2,4,5-triOCH_3, 3,4,5-triOCH_3; R_2 = H, 4-CH_3-Ph, 4-F-Ph, 4-Cl-Ph, 4-NO_2-Ph, 4-OCH_3-Ph, 2,4-diOCH_3-Ph, 3,4,5-triOCH_3-Ph.

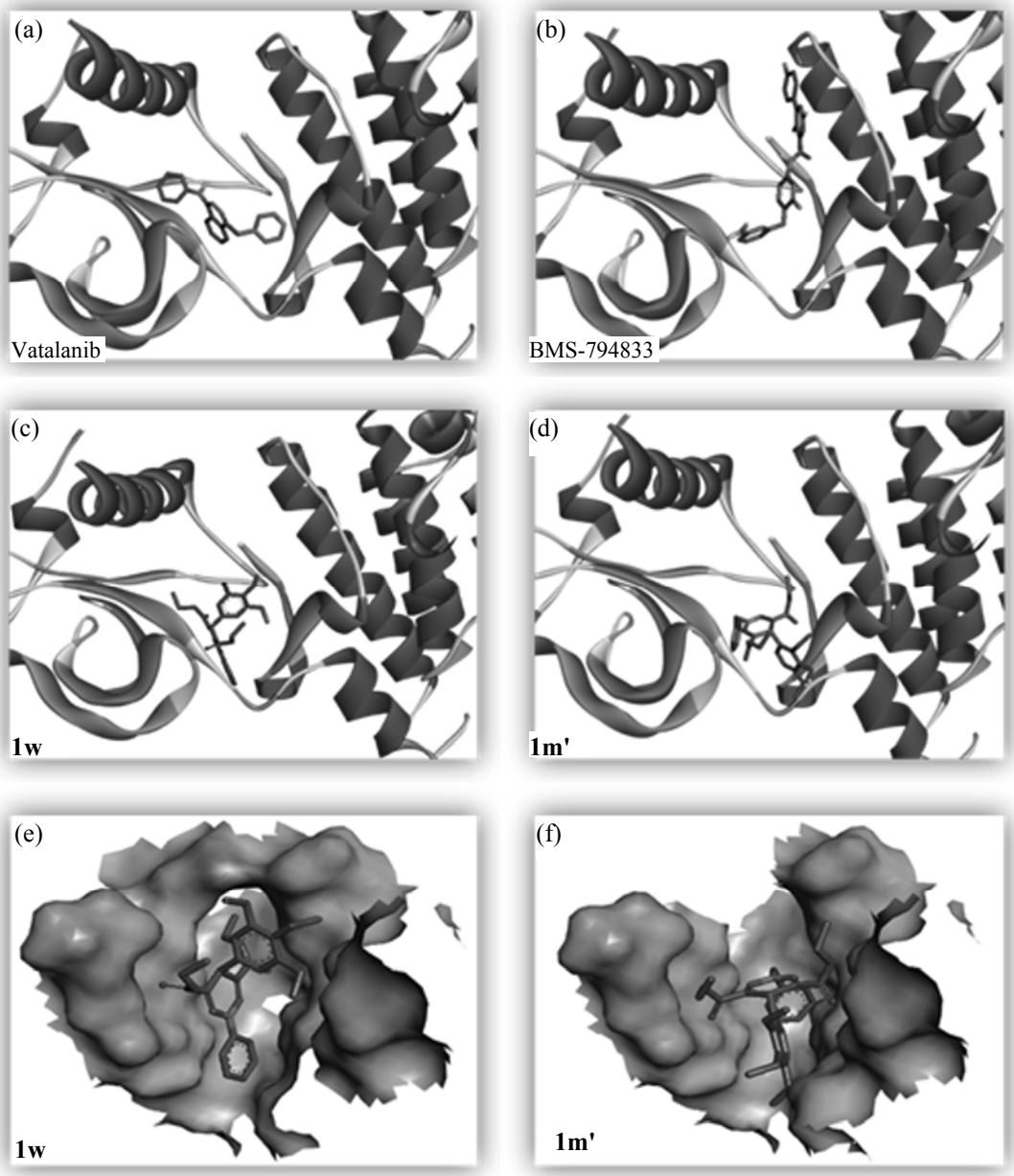

Fig. 2. The binding site of Vatalanib, BMS-794833, **1w**, and **1m'** to VEGFR-2. (a) The binding site of Vatalanib with VEGFR-2 kinase. (b) The binding site of BMS-794833 with VEGFR-2 kinase. (c) The binding site of **1w** with VEGFR-2 kinase. (d) The binding site of **1m'** with VEGFR-2 kinase. (e) 3D model of the binding site of **1w** with VEGFR-2 kinase. (f) 3D model of the binding site of **1m'** with VEGFR-2 kinase.

1, expressed as IC_{50} values, are summarized in Table 2. The products **1** exhibited promising inhibitory activeties with IC_{50} values less than 100 μM for **1w** and **1m'** that could be related to the influence of the substituents as mentioned above. It was determined that the introduction of the methoxy groups into the benzene ring increased the inhibitory activity of such compounds as **1s** (R_1 = 4-OCH_3) and **1w** (R_1 = 3,4,5-triOCH_3). Likewise, the benzyl group at the N atom increased the inhibitory activity of **1l'** (R_2 = Bn) and **1m'** (R_2 = Bn) as well. This was consistent with the molecular docking data.

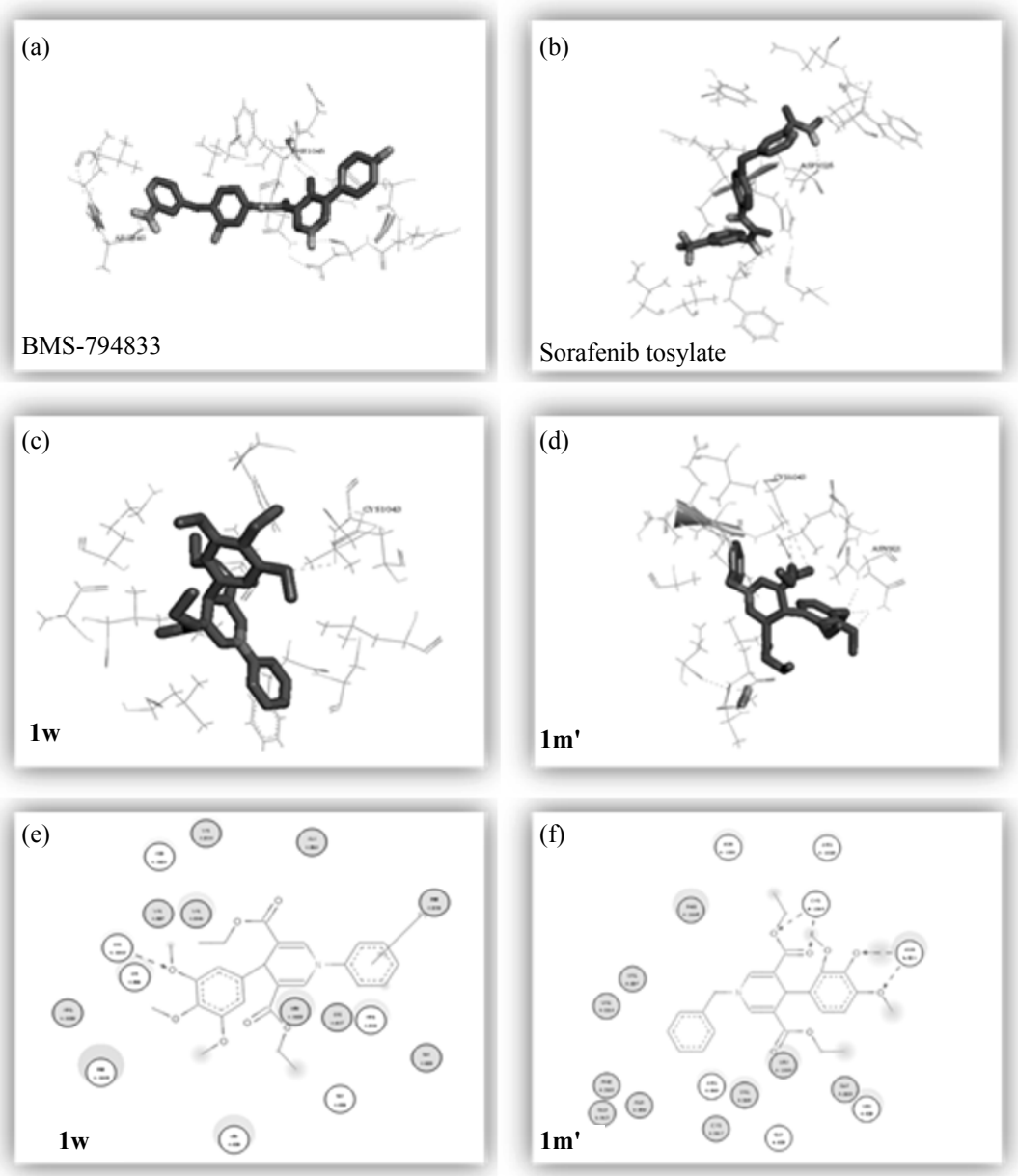

Fig. 3. The binding mode of BMS-794833, Sorafenib tosylate, **1w**, and **1m'** to VEGFR-2. (a) Interactions of BMS-794833 with VEGFR-2 kinase. (b) Interactions of Sorafenib tosylate with VEGFR-2 kinase. (c) Interactions of **1w** with VEGFR-2 kinase. (d) Interactions of **1m'** with VEGFR-2 kinase. (e) 2D diagram of interactions of **1w** with VEGFR-2 kinase. (f) 2D diagram of interactions of **1m'** with VEGFR-2 kinase.

EXPERIMENTAL

Molecular docking modeling. Molecular docking was performed using AutoDock 4.0 that used the Lamarckian genetic algorithm (LGA) for exploring the full range of ligand conformational flexibility with partial flexibility of the receptor. Three-dimensional structures of the ligands were constructed using standard bond lengths and bond angles of the GAUSSVIEW 3.09 software (Gaussian, Inc., Wallingford, CT, USA). The geometry optimization was performed by the semi-empirical AM1 method and the output files were then minimized using the density functional (DFT) method by applying the B3 LYP (Becke, Lee, Yang, and Parr) correlation function in the course of second optimization. The Gasteiger

Table 2. Biological activities of 4-aryl-1,4-dihydropyridines **1** against A549, A431 and Hep-G2 cells lines in vitro

Comp. no.	IC_{50}[a], μM			Comp. no.	IC_{50}[a], μM		
	A549	A431	Hep-G2		A549	A431	Hep-G2
1a	>200	>200	112.62	**1w**	165.91	>200	130.35
1c	159.92	127.71	>200	**1a'**	81.37	35.24	89.95
1f	144.23	166.31	>200	**1d'**	89.54	117.69	129.96
1h	101.01	78.89	70.32	**1f'**	>200	>200	133.54
1i	71.54	88.26	58.63	**1i'**	105.64	120.54	>200
1j	65.54	69.69	72.98	**1k'**	88.36	60.12	103.00
1k	80.23	90.02	58.44	**1l'**	67.23	31.39	96.49
1l	68.10	85.05	86.20	**1m'**	29.50	20.10	41.88
1m	180.76	>200	147.25	**1n'**	85.40	50.00	77.35
1n	150.50	>200	>200	**1o'**	77.18	43.25	94.60
1s	34.40	26.56	41.00	Taxol[b]	0.0006	7.359	4.855
1v	23.09	16.20	30.23				

[a] Values are the means of at least three independent experiments SD < 10%.
[b] Used as positive control.

partial charges were assigned using AutoDock Tools [15]. The crystal structure of VEGFR-2 complexed with sorafenib inhibitor (PDB code: 4ASD) was retrieved from the Brookhaven Protein Data Bank. The co-crystallized ligand was extracted from the PDB file and AutoDock Tools were used to assign polar hydrogens and Gasteiger charges. AutoGrid4 was used to create affinity grid maps for all atom types. The affinity grids enclosed a volumetric space of 50 Å with 0.375 Å spacing, centered on the coordinates x = –0.975, y = 37.541, z = 20.332. The docked conformations were generated using the LGA with an initial population size of 150 structures. The rest of parameters were set to their default values. The model analysis was performed using the ACCELRYS DS VISUALIZER 3.0 software (Accelrys, Inc., San Diego, CA, U.S.A.).

Synthesis of 1,4-bis(2,3-dihydropyrrol-5-one-4-yl)-1,3-butadienes. All chemicals were used as purchased without further purification. All solvents were reagent grade and, when necessary, purified and dried using the standard methods. Reactions progress was monitored by TLC on 0.25 mm silica gel plates (60GF-254) and visualized with UV light. The melting points were determined using an X-5 apparatus (open capillaries, uncorrected values). ^{1}H NMR spectra were measured on a Bruker ARX-400 MHz spectrometer in $CHCl_3$ using TMS as the internal standard.

General procedure for 4-aryl-1,4-dihydropyridine-3,5-dicarboxylate (1). A mixture of aromatic aldehyde (0.05 mol), ethyl propiolate (0.10 mol), amine (0.05 mol), and 5.0 mL of acetic acid was heated in a steam bath for 25 min [16, 17]. The product was crystallized from methanol : water, 4 : 1 and then recrystallized from acetone : hexane, 1 : 1.

4-Phenyl-1,4-dihydropyridine-3,5-dicarboxylate (1a). Yield 51.8%. ^{1}H NMR spectrum, δ, ppm: 1.19 t (6H, CH_2CH_3), 4.00–4.15 m (4H, CH_2CH_3), 4.89 s (1H, Ar-CH), 6.65 br.s (1H, N–H), 7.12–7.35 m (5H, Ar-H), 7.30 d (J = 5.2 Hz, 2H, C=CH).

4-(4-Hydroxyphenyl)-1,4-dihydropyridine-3,5-dicarboxylate (1c). Yield 55.4%. ^{1}H NMR spectrum, δ, ppm: 1.19 t (6H, CH_2CH_3), 4.03–4.16 m (4H, CH_2CH_3), 4.84 s (1H, Ar-CH), 6.08 br.s (1H, N-H), 6.71 d (J = 8.5 Hz, 2H, Ar-H), 7.22 d (J = 8.5 Hz, 2H, Ar-H), 7.33 d (J = 5.3 Hz, 2H, C=CH).

4-(4-Nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (1f). Yield 67.0%. ^{1}H NMR spectrum, δ, ppm: 1.19 t (6H, CH_2CH_3), 4.03–4.16 m (4H, CH_2CH_3), 5.04 s (1H, Ar-CH), 6.78 br.s (1H, N-H), 7.38 d (J = 5.8 Hz, 2H, Ar-H), 7.52 d (J = 5.8 Hz, 2H, Ar-H), 8.12 d (J = 8.5 Hz, 2H, C=CH).

4-(4-Methoxylphenyl)-1,4-dihydropyridine-3,5-dicarboxylate (1h). Yield 56.8%. ^{1}H NMR spectrum,

δ, ppm: 1.19 t (6H, CH_2CH_3), 3.75 s (3H, Ar-OCH_3), 4.03–4.13 m (4H, CH_2CH_3), 4.84 s (1H, Ar-CH), 6.28 br.s (1H, N-H), 6.77 d (*J* = 8.8 Hz, 2H, Ar-H), 7.24 d (*J* = 8.8 Hz, 2H, Ar-H), 7.30 d (*J* = 5.6 Hz, 2H, C=CH).

4-(2,4-Dimethoxylphenyl)-1,4-dihydropyridine-3,5-dicarboxylate (1i). Yield 58.0%. ^{1}H NMR spectrum, δ, ppm: 1.17 t (6H, CH_2CH_3), 3.76 s (3H, Ar-OCH_3), 3.81 s (3H, Ar-OCH_3), 3.99–4.09 m (4H, CH_2CH_3), 5.12 s (1H, Ar-CH), 6.27 br.s (1H, N-H), 6.39 s (1H, Ar-H), 6.40 d (*J* = 7.9 Hz, 1H, Ar-H), 7.16 d (*J* = 7.9 Hz, 2H, Ar-H), 7.26 d (*J* = 5.3 Hz, 2H, C=CH).

4-(2,3,4-Trimethoxylphenyl)-1,4-dihydropyridine-3,5-dicarboxylate (1j). Yield 59.5%. ^{1}H NMR spectrum, δ, ppm: 1.19 t (6H, CH_2CH_3), 3.81 s (3H, Ar-OCH_3), 3.83 s (3H, Ar-OCH_3), 3.94 s (3H, Ar-OCH_3), 4.02–4.12(m, 4H, CH_2CH_3), 5.06 s (1H, Ar-CH), 6.18 br.s (1H, N-H), 6.55 s (*J* = 8.6 Hz, 1H, Ar-H), 6.98 s (*J* = 8.6 Hz, 1H, Ar-H), 7.30 d (*J* = 5.2 Hz, 2H, C=CH).

4-(2,4,5-Trimethoxylphenyl)-1,4-dihydropyridine-3,5-dicarboxylate (1k). Yield 51.3%. ^{1}H NMR spectrum, δ, ppm: 1.18 t (6H, CH_2CH_3), 3.80 s (3H, Ar-OCH_3), 3.81 s (3H, Ar-OCH_3), 3.84 s (3H, Ar-OCH_3), 4.00–4.10 m (4H, CH_2CH_3), 5.07 s (1H, Ar-CH), 6.46 s (1H, Ar-H), 6.47 br.s (1H, N-H), 6.82 s (1H, Ar-H), 7.29 d (*J* = 5.4 Hz, 2H, C=CH).

4-(3,4,5-Trimethoxylphenyl)-1,4-dihydropyridine-3,5-dicarboxylate (1l). Yield 60.5%. ^{1}H NMR spectrum, δ, ppm: 1.22 t (6H, CH_2CH_3), 3.80 s (3H, Ar-OCH_3), 3.81 s (6H, Ar-OCH_3), 4.06–4.17 m (4H, CH_2CH_3), 4.87 s (1H, Ar-CH), 6.43 br.s (1H, N-H), 6.57 s (2H, Ar-H), 7.36 d (*J* = 5.2 Hz, 2H, C=CH).

1-Phenyl-4-phenyl-1,4-dihydropyridine-3,5-dicarboxylate (1m). Yield 42.5%. ^{1}H NMR spectrum, δ, ppm: 1.20 t (6H, CH_2CH_3), 4.04–4.18 m (4H, CH_2CH_3), 4.97 s (1H, Ar-CH), 7.15–7.48 m (10H, Ar-H), 7.67 s (2H, C=CH).

1-Phenyl-4-(4-methylphenyl)-1,4-dihydropyridine-3,5-dicarboxylate (1n). Yield 44.0%. ^{1}H NMR spectrum, δ, ppm: 1.19 t (6H, CH_2CH_3), 2.29 s (3H, Ar-CH_3), 4.04–4.17 m (4H, CH_2CH_3), 4.92 s (1H, Ar-CH), 7.05 d (*J* = 8.0 Hz, 2H, Ar-H), 7.27 d (*J* = 8.0 Hz, 2H, Ar-H), 7.24–7.48 m (5H, Ar-H), 7.65 s (2H, C=CH).

1-Phenyl-4-(4-methoxylphenyl)-1,4-dihydropyridine-3,5-dicarboxylate (1s). Yield 50.7%. ^{1}H NMR spectrum, δ, ppm: 1.21 t (6H, CH_2CH_3), 3.77 s (3H, Ar-OCH_3), 4.06–4.16 m (4H, CH_2CH_3), 4.91 s (1H, Ar-CH), 6.79 d (*J* = 8.8 Hz, 2H, Ar-H), 7.29 d (*J* = 8.8 Hz, 2H, Ar-H), 7.26–7.48 m (5H, Ar-H), 7.65 s (2H, C=CH).

1-Phenyl-4-(3,4,5-trimethoxylphenyl)-1,4-dihydropyridine-3,5-dicarboxylate (1v). Yield 60.0%. ^{1}H NMR spectrum, δ, ppm: 1.21 t (6H, CH_2CH_3), 3.80 s (6H, Ar-OCH_3), 3.81 s (3H, Ar-OCH_3), 4.11–4.18 m (4H, CH_2CH_3), 4.92 s (1H, Ar-CH), 6.60 s (2H, Ar-H), 7.26–7.48 m (5H, Ar-H), 7.67 s (2H, C=CH).

1-(4-Methylphenyl)-4-phenyl-1,4-dihydropyridine-3,5-dicarboxylate (1w). Yield 39.7%. ^{1}H NMR spectrum, δ, ppm: 1.17 t (6H, CH_2CH_3), 2.38 s (3H, Ar-CH_3), 4.04–4.15 m (4H, CH_2CH_3), 4.96 s (1H, Ar-CH), 7.14–7.27 m (5H, Ar-H), 7.16 t (*J* = 8.4 Hz, 2H, Ar-H), 7.35 t (*J* = 8.4 Hz, 2H, Ar-H), 7.62 s (2H, C=CH).

1-(4-Methoxylphenyl)-4-phenyl-1,4-dihydropyridine-3,5-dicarboxylate (1a'). Yield 56.4%. ^{1}H NMR spectrum, δ, ppm: 1.18 t (6H, CH_2CH_3), 3.83 s (3H, Ar-OCH_3), 4.02–4.16 m (4H, CH_2CH_3), 4.95 s (1H, Ar-CH), 6.95 t (*J* = 8.8 Hz, 2H, Ar-H), 7.14–7.28 m (5H, Ar-H), 7.36 t (*J* = 8.8 Hz, 2H, Ar-H), 7.55 s (2H, C=CH).

1-Benzyl-4-phenyl-1,4-dihydropyridine-3,5-dicarboxylate (1d'). Yield 46.3%. ^{1}H NMR spectrum, δ, ppm: 1.17 t (6H, CH_2CH_3), 4.01–4.11 m (4H, CH_2CH_3), 4.59 s (2H, Ar-CH_2), 4.91 s (1H, Ar-CH), 7.13–7.43 m (10H, Ar-H), 7.22 s (2H, C=CH).

1-Benzyl-4(4-hydroxyphenyl)-1,4-dihydropyridine-3,5-dicarboxylate (1f'). Yield 48.9%. ^{1}H NMR spectrum, δ, ppm: 1.18 t (6H, CH_2CH_3), 4.02–4.12 m (4H, CH_2CH_3), 4.57 s (2H, Ar-CH_2), 4.83 s (1H, Ar-CH), 6.61 d (*J* = 8.5 Hz, 2H, Ar-H), 7.10 d (*J* = 8.5 Hz, 2H, Ar-H), 7.26 s (2H, C=CH), 7.28–7.41 m (5H, Ar-H).

1-Benzyl-4(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (1i'). Yield 62.0%. ^{1}H NMR spectrum, δ, ppm: 1.10 t (6H, CH_2CH_3), 3.95–4.04 m (4H, CH_2CH_3), 4.56 s (2H, Ar-CH_2), 4.98 s (1H, Ar-CH), 6.68 d (*J* = 8.5 Hz, 2H, Ar-H), 7.23 d (2H, Ar-H), 7.27 s (2H, C=CH), 7.32 t (1H, Ar-H), 7.36 d.d (2H, Ar-H), 7.37 d (*J* = 8.6 Hz, 2H, Ar-H), 8.01 d (*J* = 8.6 Hz, 2H, Ar-H).

1-Benzyl-4(4-methoxylphenyl)-1,4-dihydropyridine-3,5-dicarboxylate (1k'). Yield 51.3%. ^{1}H NMR spectrum, δ, ppm: 1.17 t (6H, CH_2CH_3), 3.74 s (3H, Ar-OCH_3), 4.01–4.12 m (4H, CH_2CH_3), 4.58 s (2H, Ar-CH_2), 4.84 s (1H, Ar-CH), 6.68 d (*J* = 8.5 Hz, 2H, Ar-H), 7.03 d (*J* = 8.5 Hz, 2H, Ar-H), 7.22 s (2H, C=CH), 7.29–7.34 m (5H, Ar-H).

1-Benzyl-4(2,4-dimethoxylphenyl)-1,4-dihydropyridine-3,5-dicarboxylate (1l'). Yield 56.8%. ^{1}H NMR spectrum, δ, ppm: 1.15 t (6H, CH_2CH_3), 3.70 s (3H, Ar-OCH_3), 3.76 s (3H, Ar-OCH_3), 3.97–4.07 m (4H, CH_2CH_3), 4.56 s (2H, Ar-CH_2), 5.12 s (1H, Ar-CH), 6.64 d (J = 9.0 Hz, 1H, Ar-H), 6.65 s (1H, Ar-H), 7.07 d (J = 9.0 Hz, 1H, Ar-H), 7.23 s (2H, C=CH), 7.30–7.44 m (5H, Ar-H).

1-Benzyl-4(2,3,4-trimethoxylphenyl)-1,4-dihydropyridine-3,5-dicarboxylate (1m'). Yield 42.2%. ^{1}H NMR spectrum, δ, ppm: 1.17 t (6H, CH_2CH_3), 3.80 s (3H, Ar-OCH_3), 3.82 s (3H, Ar-OCH_3), 3.84 s (3H, Ar-OCH_3), 4.01–4.10 m (4H, CH_2CH_3), 4.58 s (2H, Ar-CH_2), 5.07 s (1H, Ar-CH), 6.51 d (J = 8.6 Hz, 1H, Ar-H), 6.88 d (J = 8.6 Hz, 1H, Ar-H), 7.23 s (2H, C=CH), 7.31–7.44 m (5H, Ar-H).

1-Benzyl-4(2,4,5-trimethoxylphenyl)-1,4-dihydropyridine-3,5-dicarboxylate (1n'). Yield 45.0%. ^{1}H NMR spectrum, δ, ppm: 1.18 t (6H, CH_2CH_3), 3.68 s (3H, Ar-OCH_3), 3.71 s (3H, Ar-OCH_3), 3.83 s (3H, Ar-OCH_3), 3.99–4.09 m (4H, CH_2CH_3), 4.56 s (2H, Ar-CH_2), 5.11 s (1H, Ar-CH), 6.42 s (1H, Ar-H), 6.70 s (1H, Ar-H), 7.26 s (2H, C=CH), 7.32–7.42 m (5H, Ar-H).

1-Benzyl-4(3,4,5-trimethoxylphenyl)-1,4-dihydropyridine-3,5-dicarboxylate (1o'). Yield 60.0%. ^{1}H NMR spectrum, δ, ppm: 1.21 t (6H, CH_2CH_3), 3.72 s (6H, Ar-OCH_3), 3.78 s (3H, Ar-OCH_3), 4.05–4.16 m (4H, CH_2CH_3), 4.58 s (2H, Ar-CH_2), 4.86 s (1H, Ar-CH), 6.48 s (2H, Ar-H), 7.29–7.40 m (5H, Ar-H), 7.30 s (2H, C=CH).

Biological assays. The biological activities of compounds **1** were evaluated against A549 (lung carcinoma) cells, A431 (epidermoid carcinoma) cells and Hep-G2 (hepatocellular carcinoma) cells. The cells were obtained from Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China). The cells were cultured in RPMI-1640 and maintained in a Thermo incubator (Waltham, MA) under humidified air containing 5% CO_2 and 95% air. All culture media contained 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution (10000 units of penicillin and 10 mg of streptomycin in 0.9% NaCl). The cancer cell lines were cultured in minimum essential media (MEM). Four thousand cells (per well) suspended in MEM were plated into each well of a 96-well plate and incubated for 24 h. The tested compounds were added to the culture medium at the indicated final concentrations and the cell cultures were stored for 72 h. Fresh MTT was added to each well to make the final concentration of 5 mg/mL and incubated with the cells at 37°C for 4 h. Formazan crystals were dissolved in 100 μL of DMSO for each well and the absorbance at 570 nm was measured using the multifunctional reading machine Synergy 4. All compounds were tested in triplicate for each of the cell lines. The results were expressed as average IC_{50} values of three measurements and calculated using GraphPad Prism5 software.

CONCLUSIONS

A series of 4-aryl-1,4-dihydropyridines as the potential VEGFR-2 inhibitors is presented. Molecular docking performed with the ligand binding site of VEGFR-2 indicated that 4-aryl-1,4-dihydropyridines had higher binding capacity with VEGFR-2 compared with the known inhibitors. Introduction of the methoxy groups into the benzene ring and the benzyl group at the N atom increased the binding capacity with VEGFR-2. 4-Aryl-1,4-dihydropyridines were synthesized by the reaction of aromatic aldehydes and ethyl propiolate with anilines in acetic acid. The biological activity of 4-aryl-1,4-dihydropyridines on VEGFR-2 kinase was evaluated against A549 cells, A431 cells and Hep-G2 cells using an MTT assay. Compounds **1** demonstrated promising potential inhibitory activities with IC_{50} values less than 100 μM. According to the accumulated data 4-aryl-1,4-dihydropyridines have good potential for further development as a new class of VEGFR-2 inhibitors with promising antitumor potential.

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