FULL PAPER

Diarylureas and Diarylamides with Oxazolo[5,4-*d*]pyrimidine Scaffold as Angiogenesis Inhibitors

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A series of oxazolopyrimidine-based ureas and amides were designed, synthesized, and biologically evaluated for their antiproliferative and antiangiogenic activities. These compounds were identified to exhibit inhibitory activities against human umbilical vein endothelial cells (HUVEC) *in vitro*. Among these compounds, compound **22** effectively inhibited the migration and capillary-like tube formation of human umbilical vein endothelial cells. It also exhibited a concentration-dependent inhibition on capillary sprouting from the rat aorta rings. Preliminary mechanistic studies revealed that compound **22** suppressed protein kinases activation, by decreasing PI3K and ERK 1/2 phosphorylation. These results support the further investigation of this class of compounds as potential anticancer agents.

Keywords: Oxazolopyrimidines, Ureas, Amides, Antiproliferative activity, Antiangiogenesis, Anticancer agents.

Introduction

Angiogenesis is the generation and growth of new blood vessels from the endothelium of an existing vascular network. It plays an important role in many pathological conditions, such as cancer, diabetic retinopathy, as well as numerous ischemic, inflammatory, infectious, and immune disorders [1 – 3]. The paradigm of a therapy aimed at inhibiting the formation of blood vessels, which would consequentially deprive cells and tissues of oxygen and nutrients, was born from the concept pioneered by the late *Judah Folkman* that blood vessel formation is central to the progression and maintenance of diseases which involve cellular metabolism and tissue expansion, and cancer in particular [4][5]. Starting with the hypothesis formulated by *Judah Folkman* that tumor growth is angiogenesis dependent, this area of research has a solid scientific foundation and inhibition of angiogenesis is a major area of therapeutic development for the treatment of cancer. It is a critical process in solid tumor progression because tumors of a critical size cannot grow until they develop new blood vessels to provide oxygen and nutrients. Thus, angiogenesis has been an attractive therapeutic target in the treatment of cancer in the past decades.

The angiogenesis inducers are a wide range of mediators that include many growth factors, a plethora of cytokines, bioactive lipids, matrix-degrading enzymes, and several small molecules [6]. Several approaches have been explored in the past decades to develop targeted therapies including targeting receptor tyrosine kinases (RTK) and their downstream signaling mediators, such as Ras and phosphoinositide 3-kinase (PI3K)/v-akt murine thymoma viral oncogene homolog (Akt)/mammalian target of rapamycin (mTOR) [7]. Active research in the field and subsequent clinical trials eventually resulted in US Food and Drug Administration (FDA) approval of bevacizumab for colorectal cancer in 2004 [6]. Since then, angiogenesis-targeted drugs, such as sorafenib [8], sunitinib [9], pazopanib [10], and axitinib [11], have been demonstrated as potent cancer treatment methods. Up to now, various derivatives of quinazolines [12], quinolones [13], phthalazines [14], anthranilamides [15], 2-oxindoles [16], pyrimidines [17], and pyridines [18] have been disclosed as potent inhibitors targeting angiogenesis.

Oxazolopyrimidines have an oxazole ring fused to the pyrimidine ring, which can be considered as 9-oxapurine analogs of purine. 2-Phenyl oxazolo[5,4-*d*]pyrimidines have been reported to exhibit adenosine kinase inhibition activity [19]. To further explore the biological activity of this scaffold, a series of oxazolopyrimidine-based ureas and amides were designed. In this paper, we report the synthesis and characterization of 16 oxazolopyrimidine-based ureas and amides as potent angiogenesis inhibitors.

[1]) These authors contributed equally to this work.

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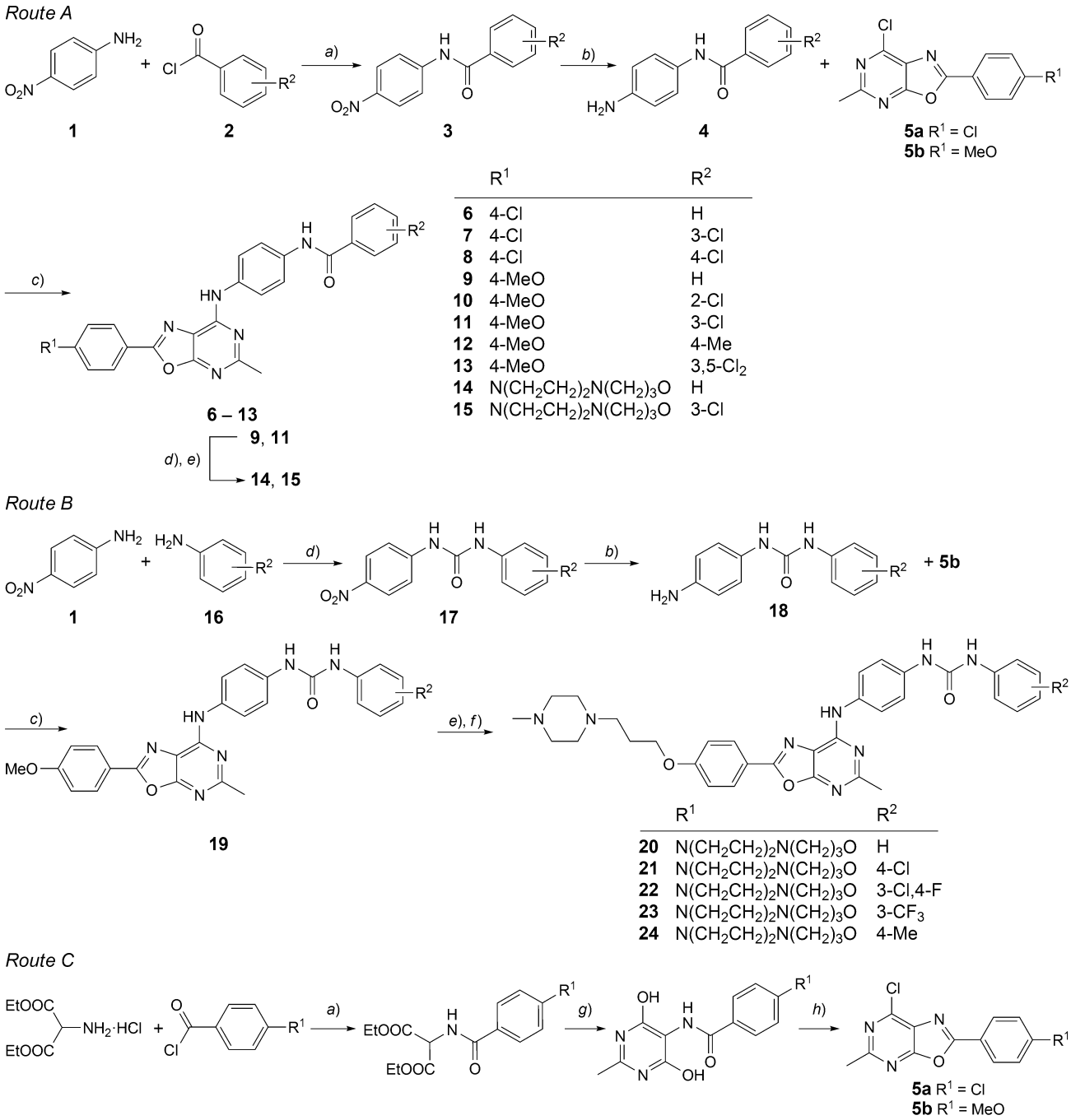
Results and Discussion

Chemistry

The synthetic method of the target compounds **6** – **15** and **20** – **24** is outlined in *Scheme 1*. Compound **3** could be prepared from the acylation of 4-nitroaniline (**1**) with aromatic acyl chlorides [20]. Compound **3** was then converted to corresponding amine **4** by reduction of Fe powder in MeOH [21]. Reaction of **4** and **5** in ^{i}PrOH afforded compounds **6** – **13** in good yields [22]. The intermediate 7-chloro-5-methyl-2-substituted phenyloxazolo [5,4-*d*]pyrimidine (**5**) was prepared as described by *Herman et al.* [23] with modification. Commercially available

Scheme 1. Synthetic route for target compounds. *Route A*: for the amide series; *Route B*: for the urea series. *Route C*: for the intermediates **5a** and **5b**.



a) TEA, CH_2Cl_2; *b*) Fe/conc. HCl, MeOH/H_2O (10:1); *c*) conc. HCl, ^{i}PrOH; *d*) BTC, TEA, toluene; *e*) BBr_3, CH_2Cl_2; *f*) 1-(3-chloropropyl)-4-methylpiperazine hydrochloride, K_2CO_3, KI, DMF, 80 °C; *g*) acetamidine hydrochloride, NaOMe/MeOH; *h*) $PhNEt_2$, $POCl_3$.

diethyl 2-aminomalonate hydrochloride was converted into diethyl 2-benzamidomalonate by reaction with aryl chloride in CH_2Cl_2. Cyclization with diethyl 2-benzamidomalonate and acetamidine hydrochloride in MeOH gave *N*-(4, 6-dihydroxy-2-methyl pyrimidin-5-yl)benzamide [24] [25], which was refluxed in $POCl_3$ and *N*,*N*-diethylaniline to afford the intermediate **5**.

The urea moiety **17** was obtained by reaction of 4-nitroaniline (**1**) with aromatic amines and triphosgene in toluene [26][27]. Then, compound **17** was converted to corresponding amine **18** by reduction of Fe powder in MeOH as in the amide series [19]. Reaction of compound **18** and **5** in ^{i}PrOH afforded intermediate **19** in good yields. Demethylation of compound **19** with BBr_3 in CH_2Cl_2 gave the demethylated intermediate [28], which was then connected with piperazine side chain to afford the product **20** – **24** [29]. Compound **14** and **15** were prepared from compound **9** and **11** in a similar way.

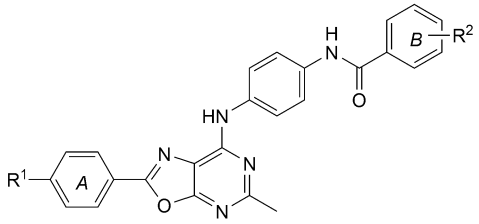
Antiproliferation Assay

The antiproliferative activity of these compounds against VEGF-induced HUVEC proliferation was determined (shown in *Table*). These compounds generally showed moderate to good inhibitory activity with IC_{50} values at 10^{-5} – 10^{-6} M (sorafenib as a positive control: 18.24 μm).

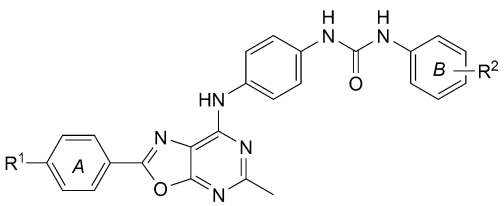
Initial efforts were directed at exploring substitutions on the 2-benzene ring. Based on the antiproliferative activity of compound **6** – **13**, we found that the substituents of the *A*-ring greatly affected the antiproliferative activity of the compounds in this series. The introduction of 4-MeO group to the *A*-ring has proven to be detrimental to antiproliferative activity. When the Cl atom was replaced by a 4-MeO group, the VEGF-HUVEC inhibitory activity was almost completely lost. This may be related to the relatively poor solubility of this series or that a 4-MeO group is not favorable for binding. Interestingly, compound **11** in this 4-MeO series showed the best antiproliferative activity with IC_{50} value of 1.28 μm. Considering that better solubility of the compounds may help improve the inhibitory activity, some hydrophilic side chains were integrated. Compound **14**, incorporated with a methylpiperazine moiety, exhibited increased VEGF-HUVEC inhibitory activity with IC_{50} value of 40.76 μM.

The replacement of the amide linker with urea resulted in a maintained or improved antiproliferative activity. A Cl atom at 3′-position on the *B* ring seems to be the optimal substituent. As in compound **7**, **11**, and **15**, the compounds maintained strong inhibitory activities (IC_{50} value of 1.31, 1.28, and 18.84 μM, resp.) comparable to or even better than that of sorafenib. However,

Table. Inhibition activity of diarylureas and diarylamides with oxazolo[5,4-*d*]pyrimidine scaffold on HUVEC proliferation



series I



series II

Entry	Compound No.	Series	R^1	R^2	HUVEC IC_{50}[μM]
1	**6**	I	4-Cl	H	62.01 ± 2.21
2	**7**	I	4-Cl	3-Cl	1.31 ± 0.34
3	**8**	I	4-Cl	4-Cl	19.31 ± 1.05
4	**9**	I	4-MeO	H	–
5	**10**	I	4-MeO	2-Cl	109.55 ± 8.56
6	**11**	I	4-MeO	3-Cl	1.28 ± 0.06
7	**12**	I	4-MeO	4-Me	–
8	**13**	I	4-MeO	3,5-Cl_2	–
9	**14**	I	[3-(4-methylpiperazin-1-yl)propyl]oxidanyl	H	40.76 ± 0.74
10	**15**	I	[3-(4-methylpiperazin-1-yl)propyl]oxidanyl	3-Cl	18.84 ± 0.25
11	**20**	II	[3-(4-methylpiperazin-1-yl)propyl]oxidanyl	H	29.10 ± 2.24
12	**21**	II	[3-(4-methylpiperazin-1-yl)propyl]oxidanyl	4-Cl	27.39 ± 0.86
13	**22**	II	[3-(4-methylpiperazin-1-yl)propyl]oxidanyl	3-Cl,4-F	12.43 ± 0.52
14	**23**	II	[3-(4-methylpiperazin-1-yl)propyl]oxidanyl	3-CF_3	37.65 ± 0.74
15	**24**	II	[3-(4-methylpiperazin-1-yl)propyl]oxidanyl	4-Me	116.43 ± 3.16
16	**25**[a])	II	4-MeO	1,3-thiazol-2-yl	–
17	Sorafenib	–	–	–	18.24 ± 1.27

[a]) In compound 25, ring *B* was replaced as a 1,3-thiazol-2-yl moiety.

compounds with a 4-Me group at the *B* ring showed decreased antiproliferative activity. Especially, compound **30** with a thiazole ring instead of the benzene ring was synthesized and evaluated. The outcome was unsatisfactory, which may indicate the benzene ring is necessary for receptor binding. Also, a 3,5-diCl substitution as in compound **13**, showed no activity in HUVEC proliferation test, which suggests that an electron-withdrawing group at 5′-postion may not be preferred. In summary, the information of SAR provided us a guideline to improve the inhibitory activity in the future structural modification.

Transwell Migration Assay

According to the data obtained on VEGF-HUVEC inhibition, compound **22** went on further evaluation considering both the antiproliferative activity and physicochemical property of the compound. We chose compound **22** instead of better acting compounds, such as compounds **7** and **11**, because compound **22** inhibited the proliferation of HUVECs in a dose-dependent manner and the dose–response curve is more '*S*'-like than that of compound **7** and **11**. Endothelial cell migration is an essential step in angiogenesis and the inhibition on this process will block the formation of new blood vessels. Therefore, compound **22** was tested for possible inhibition of endothelial cell migration in the transwell migration assay. As illustrated in *Fig. 1*, compound **22** significantly inhibited VEGF-stimulated invasion of HUVEC in a concentration-dependent manner. In the negative control group, HUVECs invaded from the upper side to the lower side of the membrane in the transwell chamber. Compound **22** reduced the VEGF-induced invasion at 14.90, 39.70, and 46.80% (percentage number of invaded cells over control) at a concentration of 0.1, 1, and 10 μM, respectively. These results demonstrate that compound **22** could suppress VEGF-induced invasion of HUVEC *in vitro*.

Tube Formation Assay

Tube formation of endothelial cell is an important process in late stages of angiogenesis. Inhibition on the formation of capillary-like tube networks will terminate the development of new blood vessels. To further characterize the antiangiogenic activity of compound **22**, we investigated the inhibitory effect of tube formation by plating HUVECs on matrigel substratum. As shown in *Fig. 2a*, reticulation of tube-like structures formed within 8 h in the negative control group. When HUVECs were exposed to compound **22**, the tube structures were destroyed dependent on compound concentration. A partial inhibition of the tube-like structure formation in which meshes were unable to form was observed when HUVECs were treated with lower concentration (5 and 2.5 μM) of compound **22**. At a concentration of 10 μM, the morphogenesis of HUVEC on the matrigel was evidently inhibited. The quantitative analysis revealed that the inhibitory rates of tube formation treated with compound **22** and sunitinib at a concentration of 5 μM were 51.2 and 88.3%,

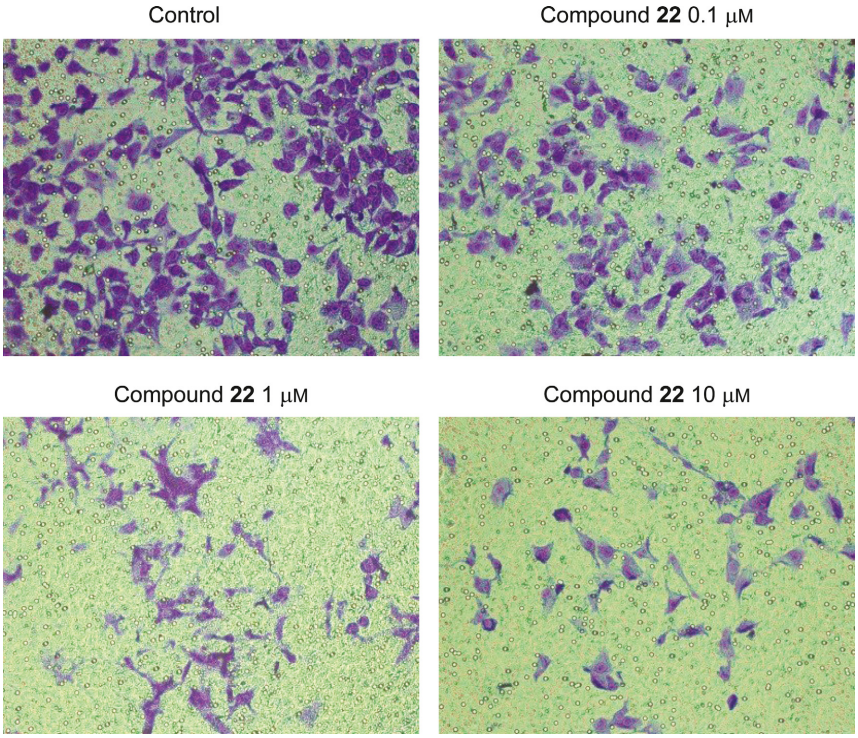


Fig. 1. Transwell migration assay. Compound **22** reduces the ability of chemotactic invasion of HUVECs.

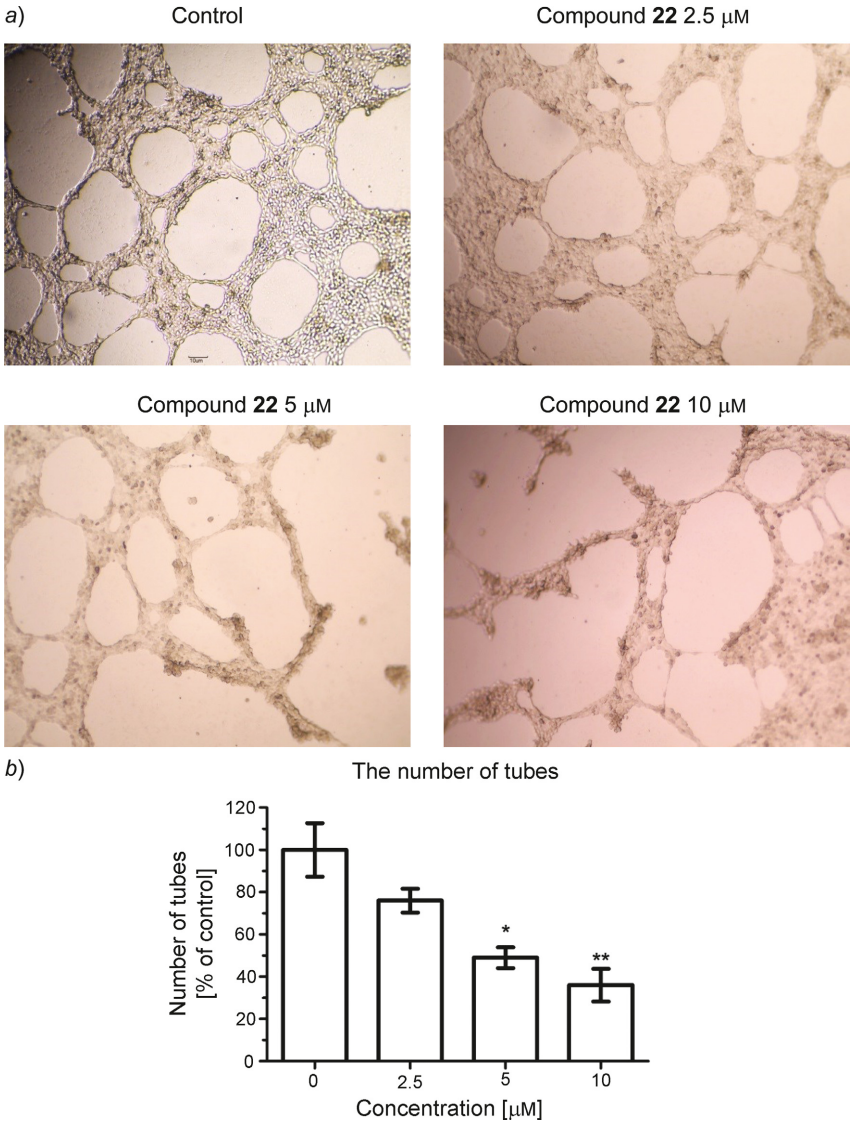


Fig. 2. Effects on the HUVECs tube formation. Compound **22** reduces VEGF-stimulated HUVEC tube formation. Data shown are expressed as means ± SD. The values represented are relative to the negative control. *$P < 0.05$ and **$P < 0.01$ *vs.* control, $n = 3$ per group.

respectively. The results demonstrated that compound **22** could effectively inhibit tube formation of HUVECs in a dose-dependent manner.

Rat Aortic Ring Assay

The rat aortic ring assay integrates the advantages of both *in vivo* and *in vitro* systems. It is an useful assay to test inhibitors in a controlled environment. In our research, rings cut from aortas were embedded and cultured in collagen gels and gave rise to endothelial outgrowths resembling microcapillary sprouts over 7 days. Compared with the negative group, the number of microvessels sprouting from the rat aortic ring was reduced to 85.05, 57.83, and 34.70% treated with compound **22** at the concentration of 2.5, 5, and 10 μM, respectively. As shown in *Fig. 3*, the number and length of microvessels were prominently decreased when treated with compound **22** at 5 and 10 μM. These results confirmed that the presence of compound **22** in the aortic outgrowth has suppressed the process of microvessels sprouting from the aortic wall *ex vivo*. In a western blot assay, compound **22** reduced the levels of activated ERK1/2 and PI3K in a concentration-dependent manner after the addition of exogenous VEGF to HUVECs. The phosphorylation of PI3K and ERK1/2 were effectively inhibited when treated with compound **22**, but the total steady-state protein levels of PI3K and ERK1/2 remained constant. These results are preliminary but provided insights for further investigations on the mechanism of compound **22**'s activity in antiangiogenesis.

Conclusions

Angiogenesis is a highly regulated process that involves a complex cascade of events, and its inhibition is now a

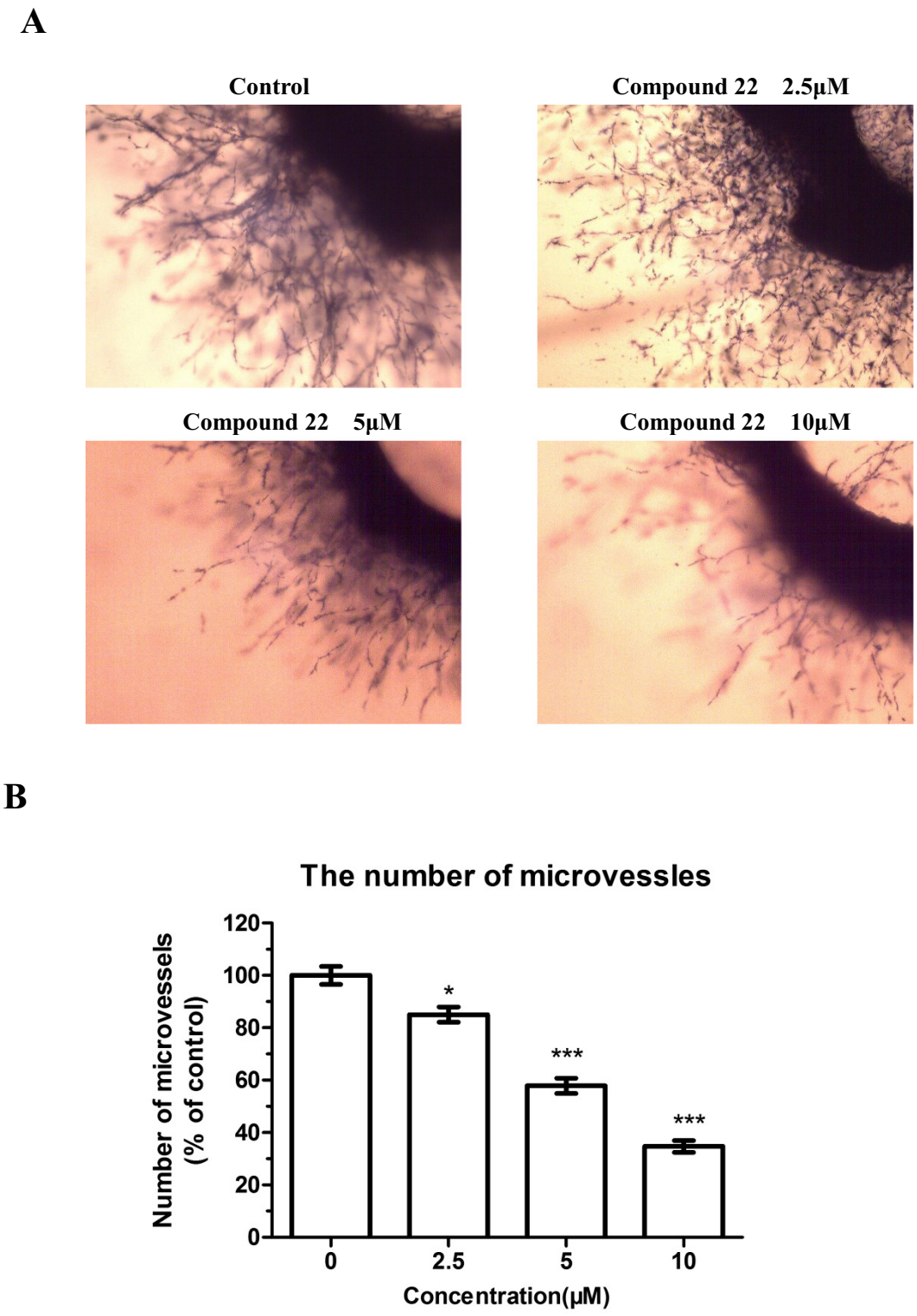


Fig. 3. Compound **22** suppresses microvessels sprouting from the rat aortic ring. Data shown are expressed as means ± SD. The values represented are relative to the negative control. *$P < 0.05$ and ***$P < 0.001$ *vs.* control, $n = 3$ per group.

well-established therapeutic strategy for cancer patients. Herein, a series of oxazolo[5,4-*d*]pyrimidine derivatives were synthesized and their antiproliferative and antiangiogenic activities were tested. Among these compounds, compound **7**, **11**, and **22** exhibited the most potent inhibitory effect on HUVEC proliferation (IC_{50} = 1.31, 1.28, and 12.43 μM, resp.). Compound **22** effectively inhibited the migration and capillary-like tube formation of

HUVEC *in vitro*. Furthermore, compound **22** also inhibited the angiogenesis in the rat aortic ring assay in a concentration-dependent manner. In conclusion, the preliminary *in vitro* antiangiogenic activities of these compounds possess potential for design of better future molecules targeting angiogenesis.

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Supplementary Material

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cbdv.201600035
Biological evaluation method, NMR and IR spectra of representative compounds were inclosed

Experimental Part

General

All reactions were monitored by HPLC using UV light for visualization. Column chromatography was performed on SiO_2 (100 – 200 mesh) made in *Qingdao Haiyang Chemical Co. Ltd*. Melting points (m.p.) were determined with an *XT-4* apparatus and are reported without correction. IR spectra were obtained with a *Nicolet Impact 410* spectrophotometer. ^{1}H-NMR spectra were collected on a *Bruker AMX 300* MHz spectrometer using $CDCl_3$ or (D_6)DMSO as the solvent with TMS as the internal reference. EI-MS: obtained on a *Shimadzu GCMS-QP2010* system. Elemental analyses were performed with an *Elementar Vario EL III* elemental analysis apparatus.

***N*-(4-Nitrophenyl)benzamide** (**3a**). Benzoyl chloride 1.38 ml (13 mmol) was added dropwise to a soln. of compound **1** (1.4 g, 10 mmol) and Et_3N (2.8 ml, 20 mmol) in anh. CH_2Cl_2 (20 ml) at 0 °C. The reaction was allowed to be stirred at r.t. for 2.5 h. The solid precipitate was filtered and washed with H_2O (2 × 20 ml). After drying under vacuum, the remaining residue was crystallized (CH_2Cl_2/hexanes, 1:3) to afford compound **3a**. Yield: 84.3%. M.p. 108 – 110 °C. EI-MS: 242 (M^+). When the same procedure for preparing compound **3a** was used, four substituted *N*-(4-nitrophenyl)benzamides were obtained.

1-(4-Nitrophenyl)-3-phenylurea (**17a**). To a soln. of triphosgene 792 mg (2.7 mmol) in toluene, *p*-nitroaniline (1105 mg, 8 mmol), and Et_3N (1.15 ml, 8 mmol) in toluene (40 ml) was added at 0 °C. After being stirred for 30 min at r.t., aniline (745 mg, 8 mmol) and Et_3N (1.15 ml, 8 mmol) in toluene (40 ml) was added to the mixture. The mixture was stirred for 15 h at 50 °C. Then, the mixture was filtered. The residue was washed with H_2O and MeOH, and dried under vacuum to give the title compound as a yellow solid. Yield: 70%. M.p. 250 – 253°. EI-MS: 257 (M^+). When the same procedure for preparing compound **17a** was used, six other substituted phenylurea compounds were obtained.

***N*-(4-Aminophenyl)benzamide** (**4a**). *N*-(4-Nitrophenyl) benzamide (**3a**; 2.04 g, 8.43 mmol) was dissolved in a mixture of MeOH (18 ml) and H_2O (2 ml). Fe powder (1.88 g) was added to it in portions. The mixture was then stirred at 85 °C for 2 h, cooled to r.t., and filtered. The filtrate was concentrated *in vacuo*. The residue was extracted with CH_2Cl_2, and the org. extract was washed with 1N NaOH, H_2O, and brine and dried (Na_2SO_4). It was then filtered and concentrated *in vacuo* to a grey solid. Purification by flash chromatography (eluting with 1% MeOH in CH_2Cl_2) yielded the pure product as an off-white solid, 1.68 g. Yield 94%. M.p. 118 – 120 °C. EI-MS: 212 (M^+). When the same procedure for preparing compound **4a** was used, aryl-substituted amides and ureas were obtained.

***N*-(4-{[2-(4-Chlorophenyl)-5-methyl[1,3]oxazolo[5,4-*d*]-pyrimidin-7-yl]amino}phenyl)benzamide** (**6**). Compounds **4a** and **5a** were added into a round-bottom bottle, and 2 ml of ^{i}PrOH and a drop of HCl were added. The mixture was refluxed for 13 h at 82 °C. It was then cooled and filtered. The residue was washed with MeOH and AcOEt, dried under vacuum to give the title compound as a pale yellow solid. Yield: 37.7%. M.p. 246 – 248 °C. IR (KBr): 3474, 3132, 1626, 1514, 1401, 1093, 991, 826, 694, 521. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.57 (*s*, 3 H); 7.52 – 7.60 (*m*, 3 H); 7.70 – 7.77 (*m*, 4 H); 7.88 (*d*, *J* = 8.9, 2 H); 7.97 (*d*, *J* = 7.0, 2 H); 8.16 (*d*, *J* = 8.5, 2 H); 10.23 (*s*, 1 H); 10.26 (*s*, 1 H). HR-ESI-TOF-MS: 456.1228 ($C_{25}H_{19}ClN_5O_2^+$, $[M + H]^+$; calc. 456.1222). When the same procedure for preparing compound **6** was used, compounds **7** – **13** were obtained.

***N*-(4-{[2-(4-Chlorophenyl)-5-methyl[1,3]oxazolo[5,4-*d*]-pyrimidin-7-yl]amino}phenyl)benzamide** (**7**). The title compound was obtained as a white solid. Yield 48.5%. M.p. 282 – 283 °C. IR (KBr): 3399, 3132, 1633, 1551, 1514, 1402, 1316, 1092. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.57 (*s*, 3 H); 7.58 (*d*, *J* = 7.7, 1 H); 7.66 – 7.75 (*m*, 5 H); 7.88 – 7.95 (*m*, 3 H); 8.02 (*s*, 1 H); 8.15 (*d*, *J* = 8.6, 2 H); 10.25 (*s*, 1 H); 10.36 (*s*, 1 H). HR-ESI-TOF-MS: 490.0832 ($C_{25}H_{18}Cl_2N_5O_2^+$, $[M + H]^+$; calc. 490.0832).

4-Chloro-*N*-(4-{[2-(4-chlorophenyl)-5-methyl[1,3]oxazolo[5,4-*d*]pyrimidin-7-yl]amino}phenyl)benzamide (**8**). The title compound was obtained as a yellow solid. Yield: 59.6%. M.p. 290 – 292 °C. IR (KBr): 3416, 3132, 1625, 1513, 1401, 1093, 1068, 516. ^{1}H-NMR (300 MHz, (D_6) DMSO): 2.57 (*s*, 3 H); 7.62 (*d*, *J* = 8.5, 2 H); 7.70 – 7.75 (*m*, 4 H); 7.89 (*d*, *J* = 8.7, 2 H); 8.00 (*d*, *J* = 8.5, 2 H); 8.15 (*d*, *J* = 8.5, 2 H); 10.25 (*s*, 1 H); 10.33 (*s*, 1 H). HR-ESI-TOF-MS: 490.0829 ($C_{25}H_{18}Cl_2N_5O_2^+$, $[M + H]^+$; calc. 490.0832).

***N*-(4-{[2-(4-Methoxyphenyl)-5-methyl[1,3]oxazolo[5,4-*d*]pyrimidin-7-yl]amino}phenyl)benzamide** (**9**). The title compound was obtained as a white solid. Yield: 40.6%. M.p. 252 – 254 °C. IR (KBr): 3351, 2843, 2605, 1657, 1512, 1419, 1322, 821, 715. ^{1}H-NMR (300 MHz, (D_6)

DMSO): 2.56 (*s*, 3 H); 3.88 (*s*, 3 H); 7.19 (*d*, *J* = 9.2, 2 H); 7.55 (*d*, *J* = 6.6, 2 H); 7.74 (*d*, *J* = 8.7, 2H), 7.87 (*d*, *J* = 9.3, 2 H); 7.97 (*d*, *J* = 7.0, 2 H); 8.11 (*d*, *J* = 8.8, 3 H); 10.13 (*s*, 1 H); 10.26 (*s*, 1 H). ESI-MS: 474.1 ([*M* + Na]$^+$). HR-ESI-TOF-MS: 452.1724 ($C_{26}H_{22}N_5O_3^+$, [*M* + H]$^+$; calc. 452.1717).

2-Chloro-*N*-(4-{[2-(4-methoxyphenyl)-5-methyl[1,3]oxazolo[5,4-*d*]pyrimidin-7-yl]amino}phenyl)benzamide (**10**). The title compound was obtained as a grey solid. Yield: 60%. M.p. > 246 °C. IR (KBr): 3418, 3133, 1628, 1583, 1513, 1402, 1306, 1254, 834. ^{1}H-NMR (300 MHz, (D_6) DMSO): 2.56 (*s*, 3 H); 3.88 (*s*, 3 H); 7.18 – 7.21 (*m*, 2 H); 7.46 – 7.59 (*m*, 6 H); 7.71 (*dd*, *J* = 1.9, 2 H); 7.87 (*d*, *J* = 8.9, 1 H); 8.11 (*d*, *J* = 8.9, 1 H); 10.49 (*s*, 1 H); 10.61 (*s*, 1 H). ESI-MS: 486.1 ([*M* + H]$^+$). HR-ESI-TOF-MS: 486.1333 ($C_{26}H_{21}ClN_5O_3^+$, [*M* + H]$^+$; calc. 486.1327).

3-Chloro-*N*-(4-{[2-(4-methoxyphenyl)-5-methyl[1,3]oxazolo[5,4-*d*]pyrimidin-7-yl]amino}phenyl)benzamide (**11**). The title compound was obtained as a white solid. Yield 70%. M.p. 269 – 271 °C. IR (KBr): 3455, 3128, 2388, 2284, 1638, 1401, 1088, 990, 838, 729, 542. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.56 (*s*, 3 H); 3.88 (*s*, 3 H); 7.19 (*d*, *J* = 8.8, 2 H); 7.58 (*t*, *J* = 7.7, 1 H); 7.66 – 7.74 (*m*, 3 H); 7.88 – 7.94 (*m*, 2 H); 8.01 (*s*, 1 H); 8.11 (*d*, *J* = 8.8, 2 H); 10.15 (*s*, 1 H); 10.35 (*s*, 1 H). ESI-MS: 508.1 ([*M* + Na]$^+$). HR-ESI-TOF-MS: 486.1327 ($C_{26}H_{21}ClN_5O_3^+$, [*M* + H]$^+$; calc. 486.1327).

***N*-(4-{[2-(4-Methoxyphenyl)-5-methyl[1,3]oxazolo[5,4-*d*]pyrimidin-7-yl]amino}phenyl)-4-methylbenzamide** (**12**). The title compound was obtained as an off -white solid. Yield: 92.5%. M.p. 275 – 276 °C. IR (KBr): 3415, 3132, 1632, 1401, 1119, 1068, 837, 520. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.40 (*s*, 3 H); 2.56 (*s*, 3 H); 3.87 (*s*, 3 H); 7.19 (*d*, *J* = 8.2, 2 H); 7.35 (*d*, *J* = 7.6, 2 H); 7.75 (*t*, *J* = 8.8, 2 H); 7.86 – 7.88 (*m*, 4 H); 8.11 (*d*, *J* = 8.0, 2 H); 10.13 (*s*, 1 H); 10.16 (*s*, 1 H). ESI-MS: 466.2 ([*M* + H]$^+$). HR-ESI-TOF-MS: 466.1873 ($C_{27}H_{24}N_5O_3^+$, [*M* + H]$^+$; calc. 466.1874).

3,5-Dichloro-*N*-(4-{[2-(4-methoxyphenyl)-5-methyl[1,3]-oxazolo[5,4-*d*]pyrimidin-7-yl]amino}phenyl)benzamide (**13**). The title compound was obtained as a gray solid. Yield: 91.6%. M.p. 285 – 286 °C. IR (KBr): 3417, 3131, 1627, 1514, 1401, 1254, 1106, 1068, 833, 518. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.56 (*s*, 3 H); 3.88 (*s*, 3 H); 7.18 (*d*, *J* = 8.8, 2 H); 7.22 (*d*, *J* = 8.6, 2 H); 7.56 (*d*, *J* = 6.3, 2 H); 7.64 (*d*, *J* = 8.6, 2 H); 7.87 (*d*, *J* = 1.9, 1 H); 8.10 (*d*, *J* = 8.9, 2 H); 10.53 (*s*, 1 H); 10.66 (*s*, 1 H). ESI-MS: 520.1 ([*M* + H]$^+$). HR-ESI-TOF-MS: 520.0943 ($C_{26}H_{20}Cl_2N_5O_3^+$, [*M* + H]$^+$; calc. 520.0938).

1-(4-{[2-(4-Methoxyphenyl)-5-methyl[1,3]oxazolo[5,4-*d*]-pyrimidin-7-yl]amino}phenyl)-3-(1,3-thiazol-2-yl)urea (**25**). The title compound was obtained as a brown solid. Yield: 69.6%. M.p. > 300 °C. IR (KBr): 3126, 2361, 1618, 1509, 1401, 1068, 834. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.53 (*s*, 3 H); 3.85 (*s*, 3 H); 7.16 (*d*, *J* = 9.0, 2 H); 7.29 (*d*, *J* = 7.4, 1 H); 7.43 (*d*, *J* = 8.0, 2 H); 7.58 (*d*, *J* = 8.6, 1 H); 7.82 (*d*, *J* = 9.0, 2 H); 8.08 (*d*, *J* = 8.5, 2 H); 9.08 (*s*, 1 H); 9.49 (*s*, 1 H); 10.04 (*s*, 1 H). ESI-MS: 474.2 ([*M* + H]$^+$).

***N*-{4-[(5-Methyl-2-{4-[3-(4-methylpiperazin-1-yl)propoxy]phenyl}[1,3]oxazolo[5,4-*d*]pyrimidin-7-yl)amino]phenyl}-benzamide** (**14**). Compound **9** (45.7 mg, 0.1 mmol) was suspended in 10 ml of CH_2Cl_2 at 0 °C and BBr_3 (0.3 mmol, in CH_2Cl_2) was added. The mixture was stirred overnight. A quantity of 5 ml of H_2O was added into the mixture at 0 °C. Then the mixture was filtered. The residue was washed with H_2O, and dried under vacuum to give as a yellow solid. Yield: 69.3%.

N-(4-{[2-(4-hydroxyphenyl)-5-methyl[1,3]oxazolo[5,4-*d*]-pyrimidin-7-yl]amino}phenyl)benzamide (70 mg, 0.15 mmol) and K_2CO_3 (55 mg, 0.4 mmol) were added into DMF (5 ml) and stirred for 20 min under N_2 atmosphere, and then 1-(3-chloropropyl)-4-methylpiperazine dihydrochloride (50 mg, 0.2 mmol) and KI (1.25 mg, 0.0075 mmol) were added. The mixture was stirred at 80 °C for 22 h. The solvent was evaporated by rotary evaporation under vacuum. The resident was chromatographed over a column of SiO_2 (CH_2Cl_2/MeOH 10:1) to give the title compound as a yellowish solid. 46.2%. M.p. 238 – 239 °C. IR (KBr): 3415, 3132, 1638, 1401, 1119, 1068, 834, 515. ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.22 (*s*, 2 H); 1.89 (*s*, 3 H); 2.47 – 2.49 (*m*, 8 H); 2.71 (*s*, 3 H); 2.87 (*m*, 2 H); 4.12 (*m*, 2 H); 7.16 (*d*, *J* = 8.8, 2 H); 7.52 – 7.58 (*m*, 3 H); 7.72 (*d*, *J* = 8.5, 2 H); 7.85 (*d*, *J* = 8.6, 2 H); 7.95 (*d*, *J* = 8.5, 2 H); 8.07 (*d*, *J* = 9.0, 2 H); 10.07 (*s*, 1 H); 10.22 (*s*, 1 H). HR-ESI-TOF-MS: 578.2870 ($C_{33}H_{36}N_7O_3^+$, [*M* + H]$^+$; calc. 578.2874).

When the same procedure for preparing compound **14** was used, compounds **15** and **20** – **24** were obtained.

3-Chloro-*N*-{4-[(5-methyl-2-{4-[(4-methylpiperazin-1-yl)-methoxy]phenyl}[1,3]oxazolo[5,4-*d*]pyrimidin-7-yl)amino]-phenyl}benzamide (**15**). The title compound was obtained as a white solid. Yield 32.7%. M.p. 251 – 253 °C. IR (KBr): 3416, 3132, 1638, 1401, 1119, 1068, 833, 515. ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.21 – 1.22 (*m*, 2 H); 2.24 – 2.26 (*m*, 2 H); 2.48 – 2.49 (*m*, 8 H); 2.54 (*s*, 3 H); 2.71 (*s*, 3 H); 4.13 – 4.14 (*m*, 2 H); 7.15 (*d*, *J* = 9.0, 2 H); 7.57 (*d*, *J* = 9.3, 1 H); 7.64 – 7.66 (*m*, 1 H); 7.71 (*d*, *J* = 9.4, 2 H); 7.84 – 7.85 (*m*, 1 H); 7.88 – 7.95 (*m*, 2 H); 7.99 – 8.01 (*m*, 1 H); 8.08 (*m*, *J* = 9.0, 2 H); 10.09 (*s*, 1 H); 10.32 (*s*, 1 H). HR-ESI-TOF-MS: 612.2485 ($C_{33}H_{35}ClN_7O_3^+$, [*M* + H]$^+$; calc. 612.2484).

1-{4-[(5-Methyl-2-{4-[(4-methylpiperazin-1-yl)methoxy]-phenyl}[1,3]oxazolo[5,4-*d*]pyrimidin-7-yl)amino]phenyl}-3-phenylurea (**20**). The title compound was obtained as a yellowish solid. Yield: 22.5%. M.p. 201 – 202 °C. IR (KBr): 3417, 3132, 1637, 1401, 1068, 833, 515. ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.22 – 1.23 (*m*, 2 H); 2.30 – 2.54 (*m*, 13 H); 2.54 (*s*, 3 H); 4.13 – 4.14 (*m*, 2 H); 6.95 (*t*, *J* = 7.1, 1 H); 7.16 (*d*, *J* = 8.8, 2 H); 7.28 (*t*, *J* = 7.6, 2 H); 7.45 (*t*, *J* = 9.2, 4 H); 7.75 (*d*, *J* = 8.8, 2 H); 8.07 (*d*, *J* = 8.6, 2 H); 8.95 (*s*, 1 H); 8.98 (*s*, 1 H); 10.00 (*s*, 1 H). HR-ESI-TOF-MS: 592.2909 ($C_{33}H_{36}N_8O_3^+$, M^+, calc. 592.2910).

1-(4-Chlorophenyl)-3-{4-[(5-methyl-2-{4-[(4-methylpiperazin-1-yl)methoxy]phenyl}[1,3]oxazolo[5,4-*d*]pyrimidin-7-yl)amino]phenyl}urea (**21**). The title compound was obtained as a yellow solid. Yield: 21.3%. M.p. 231 – 233 °C. IR (KBr): 3413, 3132, 1637, 1401, 1068, 833, 515. ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.35 (*m*, 2 H); 1.91 (*s*, 3 H); 2.32 (*s*, 5 H); 2.50 – 2.53 (*m*, 5 H); 2.54 (*s*, 3 H); 3.88 (*s*, 2 H); 6.98 (*d*, *J* = 8.8, 2 H); 7.32 (*d*, *J* = 8.9, 2 H); 7.41 (*d*, *J* = 8.9, 2 H); 7.48 (*d*, *J* = 8.9, 2 H); 7.79 (*d*, *J* = 8.8, 2 H); 7.99 (*d*, *J* = 8.7, 2 H); 8.65 (*s*, 1 H); 8.81 (*s*, 1 H); 10.36 (*s*, 1 H). ESI-MS: 627.2 ([*M* + H]$^+$). HR-ESI-TOF-MS: 626.2520 ($C_{33}H_{35}ClN_8O_3^+$, M^+; calc. 626.2521).

1-(3-Chloro-4-fluorophenyl)-3-{4-[(5-methyl-2-{4-[(4-methylpiperazin-1-yl)methoxy]phenyl}[1,3]oxazolo[5,4-*d*]-pyrimidin-7-yl)amino]phenyl}urea (**22**). The title compound was obtained as a white solid. Yield 20.7%. M.p. 213 – 215 °C. IR (KBr): 3416, 3133, 1637, 1499, 1401, 1068, 835, 517. ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.22 – 1.23 (*m*, 2 H); 1.92 (*s*, 3 H); 2.31 – 2.51 (*m*, 10 H); 2.52 (*s*, 3 H); 4.13 (*m*, 2 H); 7.18 – 7.19 (*m*, 1 H); 7.32 – 7.33 (*m*, 1 H); 7.44 – 7.45 (*m*, 1 H); 7.81 – 7.82 (*m*, 4 H); 8.09 – 8.10 (*m*, 4 H); 8.77 (*s*, 1 H); 8.95 (*s*, 1 H); 10.03 (*s*, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 165.07; 163.05; 161.85; 158.54; 153.03; 152.03; 137.69; 135.23; 134.27; 129.16; 122.11; 119.70; 118.86; 118.75; 118.67; 117.46; 117.17; 115.68; 115.13; 66.62; 54.73; 54.52; 52.55; 45.53; 26.30. ESI-MS: 645.2 ([*M* + H]$^+$). HR-ESI-TOF-MS: 644.2433 ($C_{33}H_{34}ClFN_8O_3^+$, M^+; calc. 644.2426).

1-{4-[(5-Methyl-2-{4-[(4-methylpiperazin-1-yl)methoxy]-phenyl}[1,3]oxazolo[5,4-*d*]pyrimidin-7-yl)amino]phenyl}-3-[3-(trifluoromethyl)phenyl]urea (**23**). The title compound was obtained as a white solid. Yield: 15%. M.p. 206 – 207 °C. IR (KBr): 3415, 3133, 1637, 1401, 1069, 835, 518. ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.35 (*s*, 2 H); 1.98 (*s*, 3 H); 2.28 – 2.38 (*m*, 5 H); 2.38 – 2.53 (*m*, 5 H); 2.53 (*s*, 3 H); 4.10 – 4.11 (*m*, 2 H); 7.16 – 7.16 (*m*, 2 H); 7.28 – 7.30 (*m*, 2 H); 7.43 – 7.45 (*m*, 2 H); 7.50 – 7.60 (*m*, 2 H); 7.79 – 7.81 (*m*, 2 H); 8.03 – 8.08 (*d*, *J* = 1.6, 2 H); 8.97 (*s*, 1 H); 9.31 (*s*, 1 H); 10.01 (*s*, 1 H). ESI-MS: 661.2 ([*M* + H]$^+$). HR-ESI-TOF-MS: 660.2788 ($C_{34}H_{35}F_3N_8O_3^+$, M^+; calc. 660.2784).

1-{4-[(5-Methyl-2-{4-[(4-methylpiperazin-1-yl)methoxy]-phenyl}[1,3]oxazolo[5,4-*d*]pyrimidin-7-yl)amino]phenyl}-3-(4-methylphenyl)urea (**24**). The title compound was obtained as a white solid. Yield: 16.5%. M.p. 225 – 226 °C. IR (KBr): 3416, 3126, 1636, 1509, 1401, 1256, 1068, 953, 835, 518. ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.32 – 1.34 (*m*, 2 H); 2.16 – 2.18 (*m*, 3 H); 2.23 – 2.25 (*m*, 3 H); 2.38 – 2.48 (*m*, 10 H); 2.54 (*s*, 3 H); 4.10 (*m*, 2 H); 7.06 (*d*, *J* = 8.3, 2 H); 7.14 (*d*, *J* = 9.2, 2 H); 7.31 (*d*, *J* = 8.5, 2 H); 7.39 (*d*, *J* = 8.5, 2 H); 7.76 (*d*, *J* = 8.5, 2 H); 8.05 (*d*, *J* = 9.0, 2 H); 8.52 (*s*, 1 H); 8.56 (*s*, 1 H); 9.98 (*s*, 1 H). HR-ESI-TOF-MS: 606.3074 ($C_{34}H_{38}N_8O_3^+$, M^+; calc. 606.3067).

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