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Synthesis of novel diarylamino-1,3,5-triazine derivatives as FAK inhibitors with anti-angiogenic activity

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ABSTRACT

We report herein the synthesis of novel diarylamino-1,3,5-triazine derivatives as FAK (focal adhesion kinase) inhibitors and the evaluation of their anti-angiogenic activity on HUVEC cells. Generally, the effects of these compounds on endothelial cells could be correlated with their kinase inhibitory activity. The most efficient compounds displayed inhibition of viability against HUVEC cells in the micromolar range, as observed with TAE-226, which was designed by Novartis Pharma AG. X-ray crystallographic analysis of the co-crystal structure for compound **34** revealed that the mode of interaction with the FAK kinase domain is highly similar to that observed in the complex of TAE-226.

Angiogenesis, which is regulated by the highly coordinated function of various proteins with pro- and anti-angiogenic functions is the process of new blood vessel growth from preexisting vessels. The deregulation of angiogenesis contributes to numerous disorders such as inflammatory, ischemic and immune diseases, as well as tumor growth and metastasis formation.[1] Numerous agents targeting VEGF ligands or their receptors (VEGFR), which represent one of the best validated signaling pathways in angiogenesis, have been successfully developed and tested as anti-cancer therapies.[2] Thus far, clinical benefits achieved with VEGF- and VEGFR-targeted drugs are limited by their modest efficacy and the development of resistance.[3] Therefore, other targets involved in angiogenesis need to be examined to realize the full benefits of anti-angiogenic therapy.

Focal adhesion kinase (FAK) is an ubiquitous non-receptor tyrosine–protein kinase highly conserved and localized in focal adhesions, which is activated following binding of integrins to the extracellular matrix (ECM) or upon growth factor stimulation including that mediated by VEGF. FAK has been involved in angiogenesis as an important modulator during development evidenced by the early embryonic lethality of mice engineered to harbor an endothelial specific deletion of FAK.[4] It was reported that FAK expression in endothelial cells is necessary for the formation of new blood vessels, for the stability of the vascular network and for the survival of endothelial cells.[5] Endothelial FAK-deletion in adult mice inhibited tumor growth and reduced tumor angiogenesis.[6] Furthermore, integrin–FAK signaling has been shown to activate a number of biological processes through phosphorylation and protein–protein interactions to promote tumorigenesis. FAK also plays a prominent role in tumor progression and metastasis through its regulation of both cancer cells and their microenvironments including cancer cell migration, invasion, epithelial to mesenchymal transition. Overexpression and/or increased activity of FAK is common in a wide variety of human cancers.[7] Therefore, FAK was recently proposed as a potential target in the development of anti-cancer drugs. Some FAK inhibitors have been successfully developed, which inhibited glioma, neuroblastoma and ovarian tumor growth in vivo.[8–10] Their efficacy in tumor models may be a result of their ability to potently inhibit tumor growth and tumor-associated angiogenesis.

On another side, 1,3,5-triazine ring has been often reported as an important scaffold in many chemotherapeutic agents. For example, 1,3,5-triazine derivatives containing various amino

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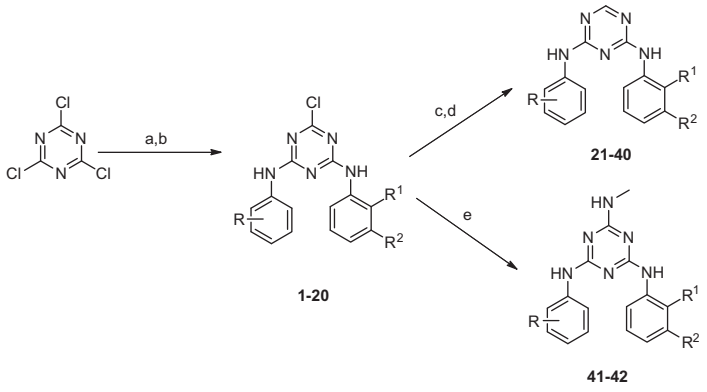
groups on the position 2, 4 or 6, such as tretamine, furazil and dioxadet, have been reported as anticancer agents.[11] Diarylaminotriazines have been claimed as ALK kinase inhibitors,[12] which may represent an effective and innovative therapy for ALCL, NSCLC, and neuroblastoma patients whose tumors harbor ALK genetic alterations.[13] Moreover, an anti-gastric ulcer agent that is commonly used in Japan, isogladine (2,4-diamino-6-(2,5-dichlorophenyl)-1,3,5-triazine), was shown to possess antiangiogenic properties in connexion with an anticancer effect.[14] The appeal of the 1,3,5-triazine core in medicinal chemistry is largely due to the ease of successive substitutions of chlorine atoms of commercially available cyanuric chloride (2,4,6-trichloro-1,3,5-triazine) with nucleophilic groups to generate a large variety of substitutions. As a part of our research program aimed at the development for new inhibitors of FAK, a series of novel diarylamino-1,3,5-triazine derivatives was prepared, according to Schemes 1 and 2.

Starting from cyanuric chloride (Scheme 1), the first chlorine was displaced by nucleophilic substitution with arylamines at −10 °C to produce the mono substituted intermediates. These were further converted to the compounds **1**–**20** through the agency of the corresponding arylamines at room temperature. These two steps could also be performed in a one pot procedure without isolating intermediates. The displacement of the last chlorine by methylamino group was more difficult and realized under heating conditions or was made by hydrogen under catalytic hydrogenation, affording the compounds **41**–**42** and **21**–**35**, respectively, in good yields. Compounds **36**–**40** were finally obtained by cleavage of their protective group.

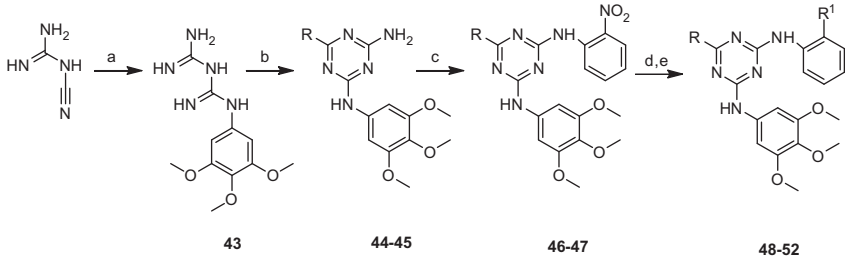
The synthesis of compounds **48**–**52** was accomplished by catalytic hydrogenation and further substitution by acetic anhydride or methanesulfonyl chloride or methyl chloroformate or dimethylcarbamoyl chloride as described in Scheme 2, from the precursors **46**–**47**, which were obtained in three steps from the microwave-assisted (MW) reaction of cyanoguanidine with arylamines and with esters and further with 1-bromo-2-nitrobenzene, using Pd-catalyzed heteroarylamination procedure.[15,16]

All compounds were evaluated for their ability to inhibit FAK kinase activity using a TR-FRET based kinase assay.[17] For detecting FAK phosphorylation activity by TR-FRET, a recombinant full length FAK protein pre-activated by Src was used with ATP and an ULight-labeled substrate poly(Glu/Tyr). Phosphorylation of the substrate was detected using an Europium-labeled phospho-specific antibody (W1024-PY20). One reported inhibitor of FAK, TAE-226, designed by Novartis Pharma AG, was included to validate the screening conditions. Under the experimental conditions, TAE-226 inhibited the activity of FAK with IC_{50} value of 7 nM (Table 1), which was similar to previously reported data.[18] As presented in Tables 1 and 2, the compounds tested demonstrated a range of potencies, clearly showing the contributions of the diarylaminotriazinic structure in terms of structure–activity relationships.

As shown in Table 1, we firstly introduced 3,4,5-trimethoxyphenylamino group on the triazine ring and a comparison of different substitutions at the position R on the triazinic ring (compounds **1**, **21**, **41** and **48**) indicated that replacement of the chlorine atom with a methylamino group for compound **41** resulted in a marginal decrease in inhibitory potency on FAK kinase activity. In contrast, removing the chlorine atom from the triazinic ring in **1** for compound **21**, displayed a about eightfold increase in inhibitory activity. Similar results were also observed for compound **22** as compared with compounds **2** and **42**. Moreover, replacement of the chlorine atom by a methyl group in compound **48** resulted in a substantial improvement in inhibitory potency as compared with **1**, but it was in the same range of **21**. This could be due to the fact that the groups R is too close to the CO of the backbone amide group of Glu-500 in the hinge (Fig. 2a), leading to steric clashes with this residue. Decreased FAK inhibitory activity might result from predisposed conformation of inhibitors less favorable to binding to the hinge region.



Scheme 1. Reagents and conditions: (a) $ArNH_2$/THF/DIEA/−10 °C; (b) $RPhNH_2$/THF/DIEA/rt; (c) H_2/Pd/THF/MeOH; (d) TFA/CH_2Cl_2; (e) CH_3NH_2/reflux.



Scheme 2. Reagents and conditions: (a) 3,4,5-trimethoxy $PhNH_2$/dioxane/MW, 90 °C, 15 min; (b) RCO_2Et/MeONa/THF/MW, 70 °C, 20 min; (c) 1-bromo-2-nitrobenzene/dioxane/$Pd(OAc)_2$/xantphos/Cs_2CO_3/MW, 150 °C, 15–30 min; (d) H_2/Pd/MeOH; (e) acetic anhydride or CH_3SO_2Cl or RCOCl/pyridine.