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Original article

3-(5-)-Amino-*o*-diarylisoxazoles: Regioselective synthesis and antitubulin activity

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ABSTRACT

A regioselective synthesis of both 5-amino- and 3-aminodiarylisoxazoles substituted with polyalkoxyaryl pharmacophores has been validated. Starting materials for the synthetic scheme were easily available from plant extracts. The targeted molecules were further tested in the phenotypic sea urchin embryo assay to identify compounds with antimitotic microtubule destabilizing activity. Structure–activity relationship studies suggested that the structural features essential for potent antiproliferative activity include: 1) 5-aminoisoxazole bridge linking biaryl substituents (rings A and B); 2) unsubstituted 5-amino group; 3) 3,4,5-methoxy substituted benzene and 4-methoxy benzene pharmacophores as rings A and B, respectively. The most potent compounds also showed strong in vitro cytotoxicity in NCI60 anticancer drug screen against a panel of 60 human cancer cell lines, including multi-drug resistant cells.

1. Introduction

Many anticancer drugs selectively target microtubules responsible for the formation of the mitotic spindle and required for proper chromosomal separation during cell division. It is generally agreed that agents affecting tubulin polymerization impair microtubule dynamics and structure, and consequently arrest cell cycle in mitosis [1–4].

Natural products of combretastatin family, namely combretastatins A-2 and A-4 (Fig. 1; CA2, CA4) are reported antimitotic compounds found in the bark of *Combretum caffrum* Kuntze (Combretaceae) [5]. It is generally recognized that these agents bind to the colchicine binding site of tubulin and affect its assembly into microtubules [6,7]. Several phosphorylated prodrugs including CA4 disodium phosphate (CA4P, Zybrestat) and combretastatin A-1 phosphate (Oxi4503) are being evaluated in the late stages of clinical trials. In addition to affecting cancer cell division, they have been found to disrupt tumor microvasculature [3,8–10].

However, despite of their therapeutic promise these agents display systemic toxicity. A significant effort has been dedicated to the discovery of synthetic combretastatins featuring potencies similar to the parent molecules but with better safety–efficacy profiles [11,12]. Examples of synthetic modifications of the ring B with diverse pharmacophores (e.g., OH, NH_2, F) are quite abundant in the literature [11,12]. At the same time, structure–activity relationship (SAR) studies of substituents in the ring A have been conducted to a lesser extent. This is presumably due to the lack of

Abbreviations: CA2, combretastatin A-2; CA4, combretastatin A-4; SAR, structure–activity relationship.

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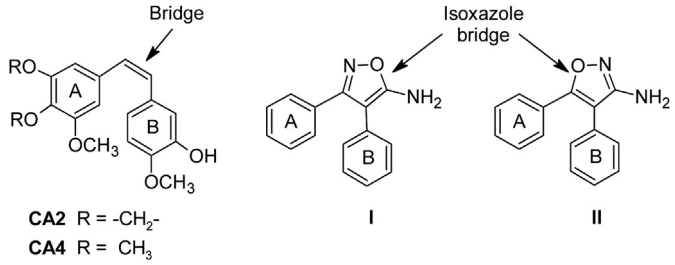


Fig. 1. Structure of combretastatins and their aminoisoxazole derivatives.

appropriate building blocks substituted with more than three alkoxy groups. It was assumed that similar modification of ring A with an additional methoxy functionality could yield potent antimitotic agents. Tetraalkoxybenzene moiety is well-represented in natural products exhibiting antimitotic properties [13–16].

A linker unit in the combretastatin derivatives is essential to lock *cis*-configuration of the biaryl pharmacophore confirmed to be necessary for the efficient interaction of a molecule with the colchicine binding site of tubulin [17,18]. As a further elaboration, five-membered heterocycles were previously reviewed to provide a nonisomerizable and metabolically stable isosteric replacement for *cis*-styrene [11,12]. Subsequently a number of novel *ortho*-diarylsubstituted four- and five-membered heterocyclic combretastatin analogs with cytotoxicity comparable to CA4 have been synthesized: 2-azetidinones [19,20], 4,5-diaryloxazoles [21], isoxasoles [22,23], diarylthiazoles [24], thiazol-2(3*H*)-ones [25], imidazol-2-ones [26], pyrazolones [27], β-lactams [28], tetrazoles [29], furanes [30], 1,2,4- [31,32], and 1,2,3-triazoles [33–38].

Recently, a series of 4,5-(polymethoxy)diaryl-3-aminopyrazole analogs of CA4 have been prepared *via* short synthetic sequences from polyalkoxybenzoic acid esters and respective aromatic acetonitriles [39,40]. Most of the key polyalkoxybenzene intermediates (benzaldehydes, benzoic acids, benzyl alcohols, aromatic acetonitriles) were easily synthesized using polyalkoxyallylbenzenes from dill and parsley seed extracts [40–42].

o-Diarylsubstituted isoxazoles were found to be the most potent antiproliferative agents [22,23,43,44]. Within this class of compounds aminoisoxazoles and their unsubstituted congeners displayed similar cytotoxic properties. For instance, cytotoxicity of some 5-amino-3,4-diaryl derivatives (Fig. 1, **I**) featuring 3,4,5-trimethoxyphenyl fragment was comparable with that of the parent CA4, when tested against five cancer cell lines [45]. These compounds inhibited purified tubulin polymerization, caused cell cycle arrest in G2/M phase, and induced apoptosis. Docking studies showed that 5-amino-3,4-diarylisoxazole overlapped well with CA4 in the colchicine binding site of tubulin [45].

As published before, 5-amino-3,4-diarylisoxazoles were synthesized by condensation of diarylcyanoketones with hydroxylamine hydrochlorides in refluxing alcohol [45]. Our attempts to follow the reported protocol yielded a mixture of regioisomers, specifically 5-amino-3,4-diaryl- (**I**) and 3-amino-4,5-diarylisoxazoles (**II**) (Fig. 1). Because of a high potency of 5-amino-3,4-diarylisoxazoles (**I**), we decided to reproduce the study by Liu et al. [45] with the aim to compare the activity of regioisomers (**I**) and (**II**), and to devise an approach to the selective synthesis of 3-amino-3,4-diarylisoxazoles (**II**).

It has been reported earlier that the cyclization of β-ketonitriles with hydroxylamine results in corresponding 5-aminoisoxazoles [46–53]. In a representative example, 5-amino-3-*tert*-butylisoxazole was prepared by reacting 4,4-dimethyl-3-oxopentanenitrile and hydroxylamine hydrochloride [46]. Notably, an alternative regioisomer, namely 3-amino-5-*tert*-butylisoxazole, was synthesized regioselectively when 4,4-dimethyl-3-oxopentanenitrile reacted with hydroxylamine in a weakly basic aqueous media [54]. It was confirmed that the initial step of this conversion involved hydroxylamine reaction with carbonyl- or nitrile-group to afford corresponding 5-amino- or 3-aminoisoxazoles depending on either nature of substituents or pH of the reaction medium [54].

Alternative regioselective routes to 5-aminoisoxazoles include addition of acetonitrile anion to α-chloroximes, condensation of α-bromoketoximines with cyanide or $LiAlH_4$-mediated rearrangement of 5-alkyl-4-cyanoisoxazoles [51,52,55,56]. To the best of our knowledge, there is only one reference to the synthesis of 3-amino-4,5-diarylisoxazoles *via* the reaction of 4-chlorophenylacetic acid amidoxime with benzoic acid chlorides [57].

Considering the potent anticancer activity of diarylaminoisoxazoles, we developed a synthesis of both 5-amino- and 3-aminodiarylisoxazoles (Fig. 1, **I**, **II**) substituted with polyalkoxyaryl pharmacophores. The targeted molecules were further tested in our phenotypic sea urchin embryo assay to identify antimitotic compounds with microtubule destabilizing activity [58]. The key phenotypic parameters that were monitored included (a) cleavage alteration and/or arrest of a fertilized egg suggestive of the antimitotic activity and (b) blastulae behavior treated with test articles immediately after hatching. Rapid embryo spinning around the animal–vegetal axis near the bottom of the vessel is an evidence of a microtubule destabilizing activity of a compound (video illustrations are available at http://www.chemblock.com). The acquisition of a specific tuberculate morphology of arrested eggs could be considered as additional indirect proof of the ability of a molecule to destabilize microtubules [58]. Several compounds with strong antimitotic antitubulin effects in the sea urchin embryo assay were further selected for cytotoxicity assessment in the NCI60 human tumor cell line anticancer drug screen.

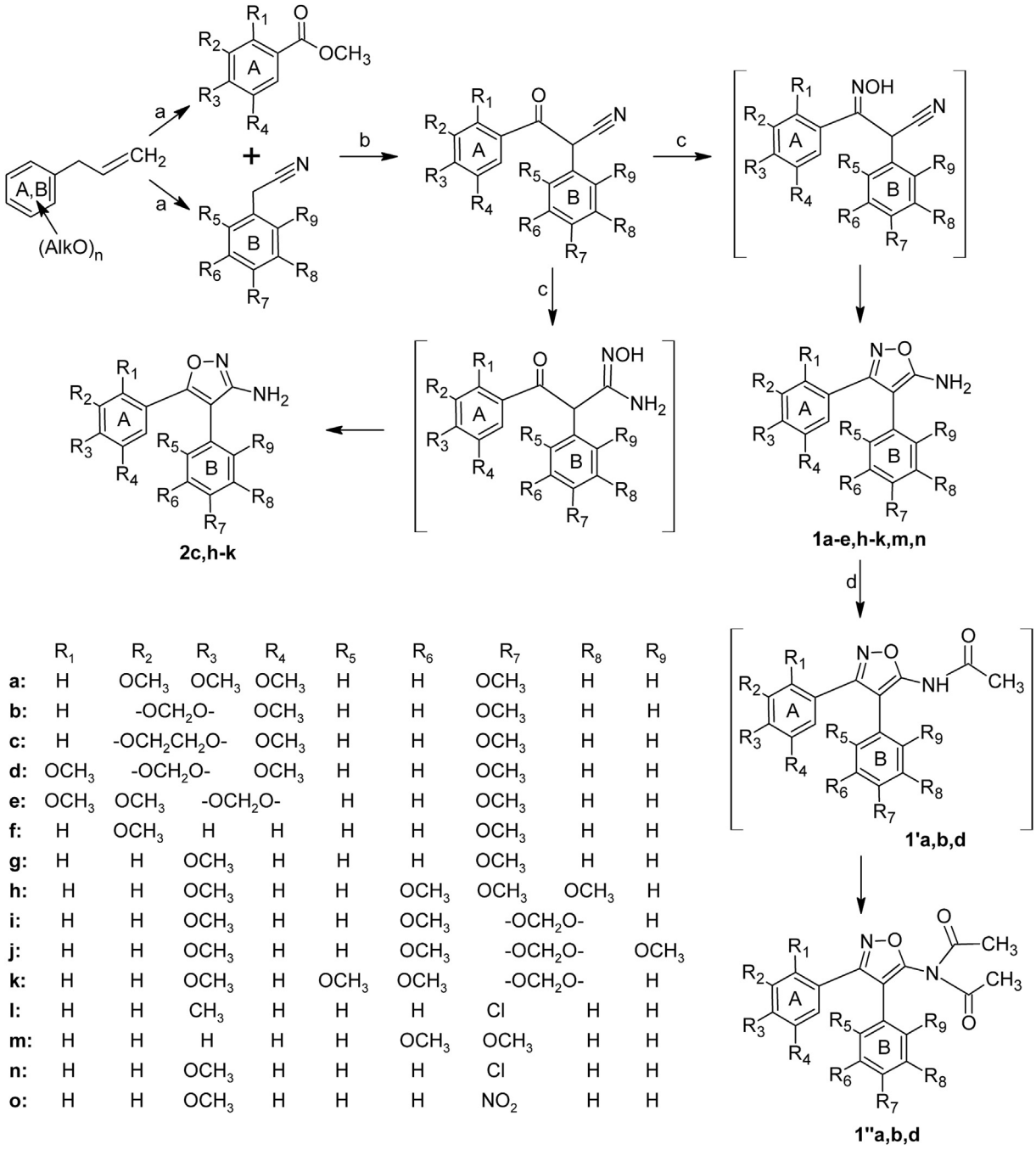
2. Results and discussion

2.1. Chemistry

The original route based on chlorooximes was not suitable for the synthesis of polyalkoxyaryl 5-amino-3,4-diarylisoxazoles primarily due to the chlorination of the electron rich benzene ring. In order to address this issue, we investigated condensation of cyanoketones with hydroxylamines. In our hands, the reaction of diarylcyanoketones with hydroxylamine hydrochlorides in refluxing alcohol resulted in two regioisomers, namely 5-amino-3,4-diarylisoxazoles **1a–e**, **1h–k**, **1m**, and **1n** and 3-amino-4,5-diarylisoxazoles **2c**, **2h–j**, and **2k** (Scheme 1). In most cases, the ratio of regioisomers 5-amino/3-amino was in the 1:1–1:1.5 range. Individual compounds were isolated by column chromatography. Notably, reaction of 5-aminoisoxazoles with Ac_2O in pyridine resulted in diacylisoxazoles **1″a**, **1″b**, and **1″d**. The respective intermediate mono-acyl derivatives **1′a**, **1′b**, and **1′d** were not separated.

Signals of the NH_2-group corresponding to regioisomers **1** and **2** were well separated (δ in DMSO was 5.2–5.3 ppm for 3-aminoisoxazoles and 6.4–6.9 ppm for 5-aminoisoxazoles, respectively). In addition, 3-aminoisomers usually had melting point values up to 15–20 °C higher than respective 5-amino derivatives. Structure of 3-aminoisoxazoles were further confirmed by an independent crystallography data.

The molecular structure of **2c** was unambiguously determined by a single crystal X-ray diffraction study. Compound **2c** was a 3-amino-4,5-diarylisoxazole derivative, which crystallized in the triclinic space group *P*-1 featuring two independent molecules per crystallographic unit (Fig. 2). The geometry of these two



	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9
a:	H	OCH_3	OCH_3	OCH_3	H	H	OCH_3	H	H
b:	H	$-OCH_2O-$		OCH_3	H	H	OCH_3	H	H
c:	H	$-OCH_2CH_2O-$		OCH_3	H	H	OCH_3	H	H
d:	OCH_3	$-OCH_2O-$		OCH_3	H	H	OCH_3	H	H
e:	OCH_3	OCH_3	$-OCH_2O-$		H	H	OCH_3	H	H
f:	H	OCH_3	H	H	H	H	OCH_3	H	H
g:	H	H	OCH_3	H	H	H	OCH_3	H	H
h:	H	H	OCH_3	H	H	OCH_3	OCH_3	OCH_3	H
i:	H	H	OCH_3	H	H	OCH_3	$-OCH_2O-$		H
j:	H	H	OCH_3	H	H	OCH_3	$-OCH_2O-$		OCH_3
k:	H	H	OCH_3	H	OCH_3	OCH_3	$-OCH_2O-$		H
l:	H	H	CH_3	H	H	H	Cl	H	H
m:	H	H	H	H	H	OCH_3	OCH_3	H	H
n:	H	H	OCH_3	H	H	H	Cl	H	H
o:	H	H	OCH_3	H	H	H	NO_2	H	H

Scheme 1. Synthesis of mixtures of 5-amino- and 3-aminodiarylisoxazoles by condensation of cyanoketones with hydroxylamines. Reagents and conditions: (a) Refs. [40–42]; (b) Ref. [40]; (c) $NH_2OH{\cdot}2HCl$–NaOAc, EtOH, reflux, 5 h; (d) Py–Ac_2O, r.t., 24 h.

independent molecules was very similar. Specifically, the isoxazole ring was planar with all bond lengths matching conventional O–N, O–C, C–C, N=C and C=C bonds reported for this heterocyclic system (Table S1, Supplementary data). Hydrogen bonds and crystal packing for **2c** and other details are presented in Table S2 and Fig. S1 (Supplementary data). Aryl substituents (methoxyphenyl and 5-methoxy-1,4-benzodioxane) were not coplanar to the central isoxazole ring. Nitrogen atoms of the amino groups adopted a trigonal-planar configuration. Notably, the amino groups were found to be within the planes of the isoxazole ring, and the methoxy groups were aligned with the benzene planes. Crystallographic data for **2c** have been deposited with the Cambridge Crystallographic Data Center (CCDC 915647).

We attempted to prepare 3-aminoisoxazoles *via* the reaction of phenylacetic acid amidoximes with benzoyl chlorides in pyridine (Scheme 2). In a representative example, condensation of 4-chlorophenylacetic acid amidoxime with tolyl chloride according to the published procedure [57] did afford the anticipated 3-aminoisoxazole derivative **4l** albeit in a moderate yield (43%). Moreover, similar protocol conducted with amidoxime derivatives of electron-rich arylacetic acid (ex., CH_3O-substituted) **3p–s** furnished benzyloxadiazoles **5a–f**, **h**, and **t**. Formation of benzyloxadiazoles **5** from phenylacetic acid aldoximes **3** was unequivocally confirmed by the high-resolution NMR experiments including heteronuclear coupling observation: ^{13}C (SF = 125.76), (^1H–^{13}C) HSQC and (^1H–^{13}C)HMBC for **5c** and **h**. Traces of 3-aminoisoxazole

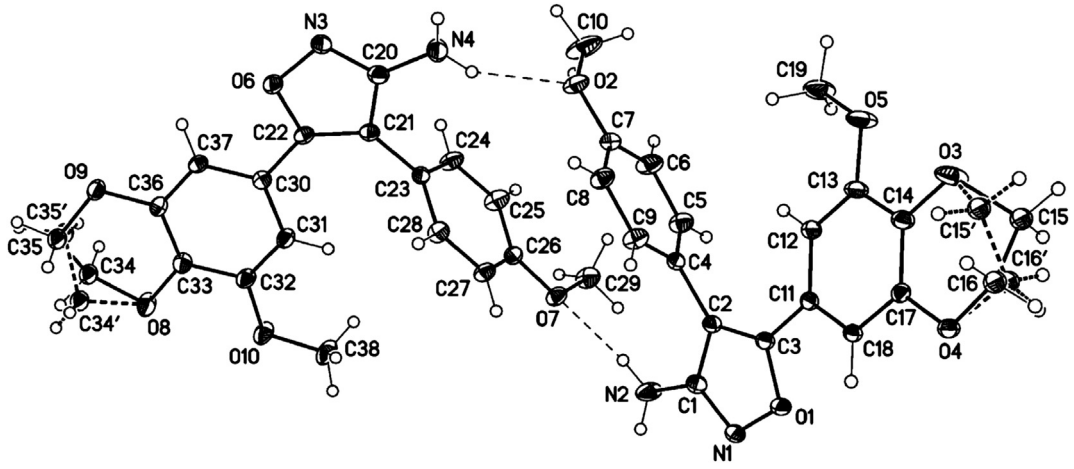


Fig. 2. Molecular structure of 3-aminoisoxazole **2c**. Two crystallographically independent molecules are presented. The alternative positions of the disordered $-CH_2-CH_2-$ fragments are shown by thick dashed lines. Thin dashed lines indicate the intermolecular N–H···O hydrogen bonds.

4h (ca. 10%) were detected in a single instance, namely upon condensation of **3q** with *p*-methoxybenzoylchloride (Scheme 2).

As a next step, we studied the reaction of equimolar 4-methoxyphenylacetic acid amidoxime **3p** with *m*-methoxybenzoyl chloride to yield the intermediate *O*-benzoyl derivative **6f** (Scheme 2). Optimization of reaction conditions allowed for the development of a regioselective synthesis of 3-aminoisoxazoles. Depending on the reaction time and temperature (20–60 °C), addition of a 3–4 fold excess of Ac_2O afforded both mono- (**2′f**) and di-*N*-acetyl (**2″f**) derivatives of 3-aminoisoxazoles in modest yields. Similar reaction conducted in the refluxed pyridine furnished oxadiazole **5f** (ca 25%) in addition to the products listed above. Treatment with a two-fold excess of AcCl at room temperature for 1 h improved yields of monoacetyl 3-aminoisoxazoles **2′a**–**h**, **2′n**, and **2′o** (50–60%), respective oxadiazoles were not detected in the reaction mixtures. Structures of *N*-acyl-3-aminoisoxazoles were confirmed by high resolution NMR-experiments: (^{1}H–^{1}H) NOESY for **2′a** and (^{1}H–^{13}C)HMBC and ^{13}C (SF = 125.76) for **2′h**. Longer reaction time (5–15 h) led to the extensive formation of side products and lower yields of the targeted 3-aminoisoxazoles. For the derivative **6d**, we isolated intermediate product, namely *N*-acyl-*O*-benzoylamidoxime **7d**, which was successfully converted to the targeted 3-acylaminoisoxazole **2′d** upon heating in pyridine in a 60% yield.

2.2. Antimitotic microtubule destabilizing activity in the phenotypic sea urchin embryo assay

The antimitotic and microtubule destabilizing effects of synthesized aminoisoxazoles were investigated using the phenotypic sea urchin embryo assay [58]. The results are presented in Table 1. CA2 and CA4 served as reference compounds.

Table 1 illustrates that 5-aminoisoxazoles **1a**–**c**, **1e**, **1h**, **1m**, **1″a**, **1″b**, and **1″d** and 3-aminoisoxazoles **2c** and **2h** exhibited well-defined cleavage alteration, cleavage arrest, and embryo spinning with EC values ranging from 0.0005 to 0.5 μM, providing evidence for their antimitotic microtubule destabilizing activity. The data correlated well with the literature, namely compounds **1a** and **1″a** inhibited purified tubulin polymerization, and **1″a** also caused G2/M arrest typical for tubulin targeting agents [45]. Among 5-amino derivatives, molecules **1a**, **1c**, and **1h** exhibited activity similar to that of CA2 and CA4 in the sea urchin embryo assay, whereas compound **1b** was determined to be even more potent. 5-Aminoisoxazole **1i** could be considered as an antitubulin agent as well, because at 2 μM it caused formation of tuberculate eggs, although it did not induce embryo spinning. Benzyloxadiazoles **5a**–**f**, **h**, and **t** were inactive up to 4 μM concentration (data not shown).

2.2.1. Structure–activity studies in the sea urchin embryo assay. Modifications of aminoisoxazole bridge

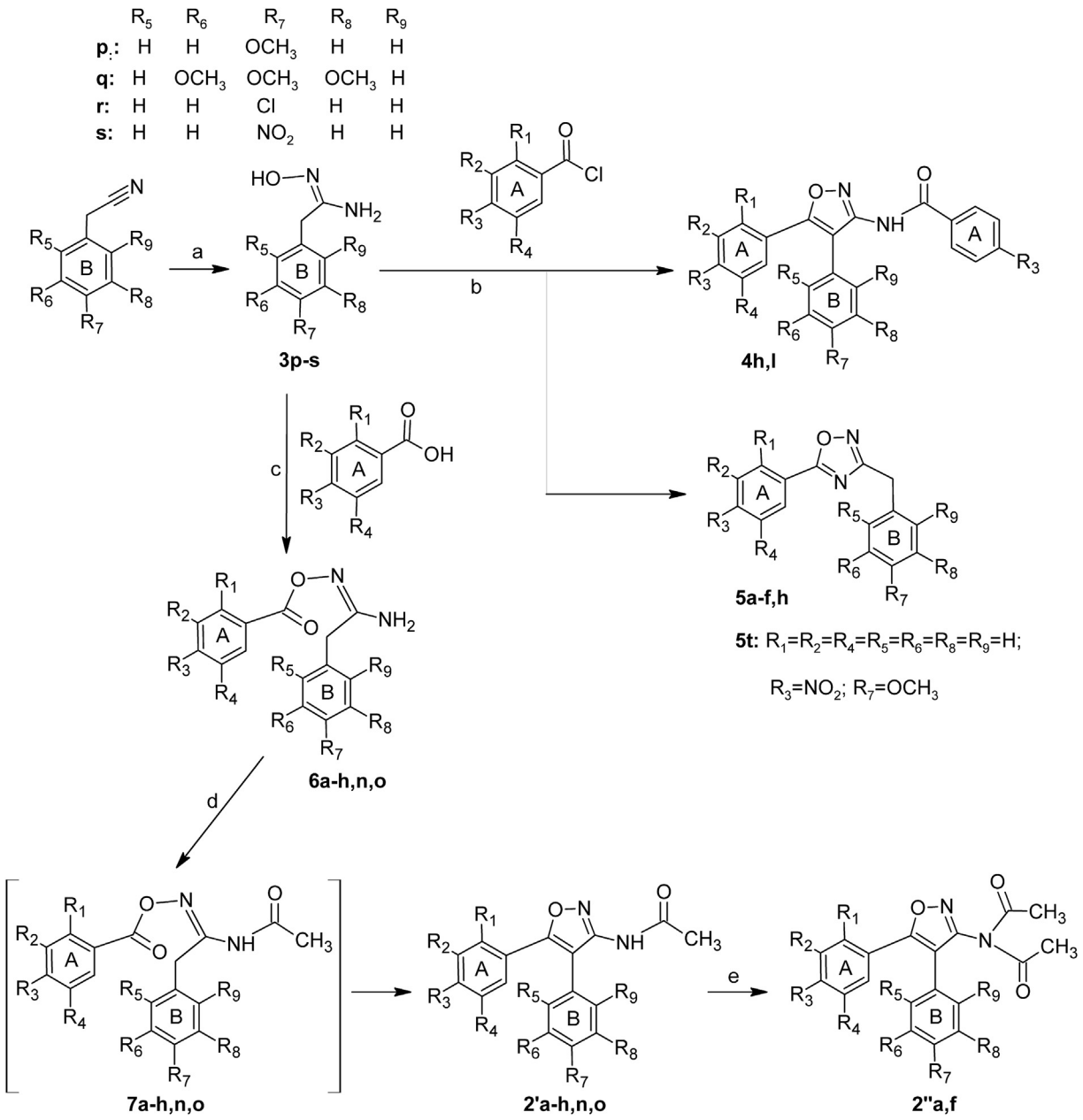
Comparing the activity of regioisomers showed that 5-aminoisoxazoles were consistently more potent antimitotic agents than respective 3-aminoisoxazoles (by ca. 2-fold, compare **1c** and **2c**, **1h** and **2h**, **1i** and **2i**). For 3-aminoisoxazoles, *N*-acetylation yielded inactive molecules (compare **2c** and **2′c**; **2h** and **2′h**). Other *N*-monoacetyl-3-aminoisoxazoles **2′a**, **2′b**, **2′d**–**g**, **2′n**, and **2′o** had no effect on the sea urchin embryo development as well up to 4 μM concentration. Similarly, *N*-benzoyl compound **4h** did not cause any developmental abnormalities. Moreover, *N*,*N*-diacetyl-5-aminoisoxazoles were consistently less active than the respective amino derivatives (compare **1a** and **1″a**, **1b** and **1″b**). For tetra-substituted 5-aminoisoxazoles, diacetylation increased the antimitotic effect (compare **1d** and **1″d**). It is worth noting that isoxazoles **1a** and **1b** were considerably more active than their corresponding pyrazole analogs (compounds **12a** and **12b**, [40]).

2.2.2. Substitutions in the rings A and B

In the 5-aminoisoxazoles series with *p*-methoxy ring B, the effect on sea urchin embryos in relation to the structure of ring A decreased in the following order: 3,4-methylenedioxy-5-methoxy phenyl (**1b**) > 3,4,5-trimetoxyphenyl (**1a**) ≈ 3,4-ethylenedioxy-5-methoxyphenyl (**1c**) > 2,3-dimethoxy-4,5-methylenedioxyphenyl (**1e**) > 2,5-dimethoxy-3,4-methylenedioxyphenyl (**1d**). These data suggested that tetraalkoxy substitution in the ring A reduced the antimitotic effect of a molecule. Similarly, activity of compounds endowed with tetraalkoxysubstituted ring B and *p*-methoxy ring A (**1j**, **1k**, **2j**, and **2k**) was markedly reduced.

Pharmacophore 'swapping' (exchange of rings A and B) in the regioisomers of 5-aminoisoxazoles yielded mixed results. For example, the antimitotic effects of compounds **1a** vs **1h** and **1d** vs **1j** were comparable. In other cases, molecules with monomethoxy phenyl substituent for the ring A were considerably less active (compare **1b** and **1i**, **1e** and **1k**).

In summary, structure–activity relationship studies further suggested that the structural features essential for yielding potent antimitotic compounds with microtubule destabilizing properties



Scheme 2. Regioselective synthesis of 3-aminoisoxazoles. Reagents and conditions: a) EtOH–H_2O, reflux, 5 h; b) Py, reflux, 1 h; c) CH_3CN, CDI, r.t., 8 h; d) AcCl, Py, r.t., 1.5 h; e) Ac2O, Py, 60 °C, 24 h.

include: 1) 5-aminoisoxazole bridge linking biaryl substituents (rings A and B); 2) unsubstituted 5-amino group; 3) 3,4,5-methoxy substituted benzene and 4-methoxy benzene pharmacophores as rings A and B, respectively.

2.3. *In vitro cancer cell growth inhibition*

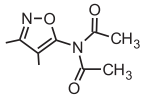
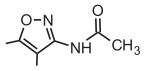
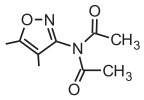
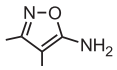
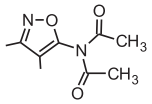
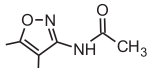
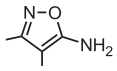
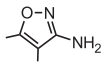
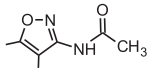
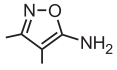
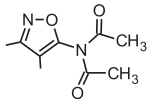
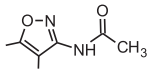
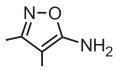
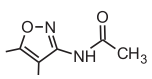
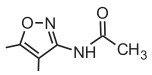
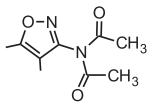
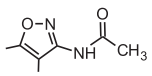
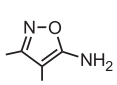
Compounds **1a**–**c**, **1e**, **1i**, and **2i** were further selected for evaluation in the NCI-60 human tumor cell line anticancer drug screen. The results are presented in Table 2 and Table S3 (Supplementary data).

Non-small cell lung carcinoma (NCI-H522), leukemia (SR), melanoma (MDA-MB-435), and colon carcinomas were the most sensitive cell lines towards 5-aminoisoxazoles **1a**–**c** (Table S3, Supplementary data). These compounds affected growth of a number of cancer cell lines with $GI_{50} < 10$ nM. In addition, molecules **1a** and **1c** inhibited cell growth of COLO 205, HT-29 (both colon cancer), and 786-0 (renal cancer) significantly more than CA4 (Table S3, Supplementary data).

Generally, the most potent compounds identified in the sea urchin embryo assay (**1a**–**c**) showed strong cytotoxicity against human cancer cells with GI_{50} in a low nanomolar concentration range. Interestingly, all molecules tested in NCI60 screen were more potent against P-glycoprotein-overexpressing multi-drug resistant cell line NCI/ADR-RES than against the parent ovarian cancer cell line OVCAR-8 (Table 2).

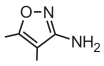
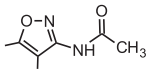
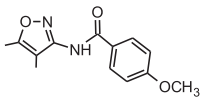
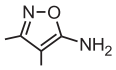
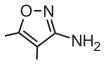
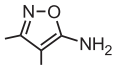
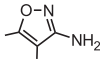
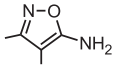
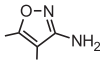
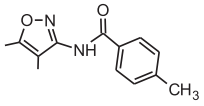
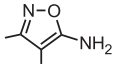
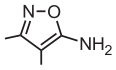
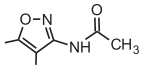
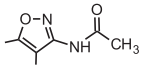
It is worth noting that methylenedioxy-methoxy derivative **1b** showed higher activity than the respective thrimethoxyphenyl analog **1a** (ca. 4-fold) in the sea urchin embryo assay, whereas the cytotoxicity data were reversed, namely, **1a** was more active than

Table 1
Effects of 3- and 5-aminoisoxazoles on sea urchin embryo.

Compd	Bridge	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	Sea urchin embryo effects, EC (μM)[a]		
											Cleavage alteration	Cleavage arrest	Embryo spinning
CA2											0.002	0.01	0.05
CA4											0.002	0.01	0.05
1a		H	OCH_3	OCH_3	OCH_3	H	H	OCH_3	H	H	0.002	0.005	0.5
1″a		H	OCH_3	OCH_3	OCH_3	H	H	OCH_3	H	H	0.05	0.2	1
2′a		H	OCH_3	OCH_3	OCH_3	H	H	OCH_3	H	H	>4	>4	>4
2″a		H	OCH_3	OCH_3	OCH_3	H	H	OCH_3	H	H	ND[b]	ND[b]	ND[b]
1b		H	$-OCH_2O-$		OCH_3	H	H	OCH_3	H	H	0.0005	0.002	0.05
1″b		H	$-OCH_2O-$		OCH_3	H	H	OCH_3	H	H	0.02	0.1	0.5
2′b		H	$-OCH_2O-$		OCH_3	H	H	OCH_3	H	H	>4	>4	>4
1c		H	$-OCH_2CH_2O-$		OCH_3	H	H	OCH_3	H	H	0.002	0.01	0.1
2c		H	$-OCH_2CH_2O-$		OCH_3	H	H	OCH_3	H	H	0.005	0.02	0.5
2′c		H	$-OCH_2CH_2O-$		OCH_3	H	H	OCH_3	H	H	>4	>4	>4
1d		OCH_3	$-OCH_2O-$		OCH_3	H	H	OCH_3	H	H	2	>5	>5
1″d		OCH_3	$-OCH_2O-$		OCH_3	H	H	OCH_3	H	H	0.5	2	5
2′d		OCH_3	$-OCH_2O-$		OCH_3	H	H	OCH_3	H	H	>4	>4	>4
1e		OCH_3	OCH_3	$-OCH_2O-$		H	H	OCH_3	H	H	0.02	0.2	2
2′e		OCH_3	OCH_3	$-OCH_2O-$		H	H	OCH_3	H	H	>4	>4	>4
2′f		H	OCH_3	H	H	H	H	OCH_3	H	H	>4	>4	>4
2″f		H	OCH_3	H	H	H	H	OCH_3	H	H	ND[b]	ND[b]	ND[b]
2′g		H	H	OCH_3	H	H	H	OCH_3	H	H	>4	>4	>4
1h		H	H	OCH_3	H	H	OCH_3	OCH_3	OCH_3	H	0.002	0.01	0.1

(*continued on next page*)

Table 1 (*continued*)

Compd	Bridge	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	Sea urchin embryo effects, EC (μM)[a]		
											Cleavage alteration	Cleavage arrest	Embryo spinning
2h		H	H	OCH_3	H	H	OCH_3	OCH_3	OCH_3	H	0.004	0.02	0.2
2′h		H	H	OCH_3	H	H	OCH_3	OCH_3	OCH_3	H	>4	>4	>4
4h		H	H	OCH_3	H	H	OCH_3	OCH_3	OCH_3	H	>4	>4	>4
1i		H	H	OCH_3	H	H	OCH_3	$-OCH_2O-$		H	0.2	2	>5
2i		H	H	OCH_3	H	H	OCH_3	$-OCH_2O-$		H	0.2	>4	>10
1j		H	H	OCH_3	H	H	OCH_3	$-OCH_2O-$		OCH_3	4	>4	>4
2j		H	H	OCH_3	H	H	OCH_3	$-OCH_2O-$		OCH_3	>4	>4	>4
1k		H	H	OCH_3	H	OCH_3	OCH_3	$-OCH_2O-$		H	>4	>4	>4
2k		H	H	OCH_3	H	OCH_3	OCH_3	$-OCH_2O-$		H	4	>4	>4
4l		H	H	CH_3	H	H	H	Cl	H	H	ND[b]	ND[b]	ND[b]
1m		H	H	H	H	H	OCH_3	OCH_3	H	H	0.5	10	10
1n		H	H	OCH_3	H	H	H	Cl	H	H	>4	>4	>4
2′n		H	H	OCH_3	H	H	H	Cl	H	H	>4	>4	>4
2′o		H	H	OCH_3	H	H	H	NO_2	H	H	>4	>4	>4

[a] The sea urchin embryo assay was conducted as described previously [58]; fertilized eggs and hatched blastulae were exposed to 2-fold decreasing concentrations of compounds; duplicate measurements showed no differences in effective threshold concentration (EC) values.
[b] ND: not determined.

1b. This relationship was also observed for combretastatins (CA4 and CA2) [42] and 4-aza-podophyllotoxins [59]. Whereas this discrepancy deserves a detailed investigation, it could be attributed to the fact that cultured cancer cells usually divide every 20–24 h as compared to a much shorter mitotic cycle (35–45 min) of the sea urchin blastomeres. As a result, microtubule destabilizing agents are likely to affect predominantly interphase microtubules in cancer cells. Their main target in the sea urchin embryo could be the mitotic spindle. These two types of microtubules may exhibit different sensitivity when treated with an antitubulin agent.

It was reported that 5-aminoisoxazoles **1a** and **1h** [45] and respective 3-aminopyrazoles [39] displayed comparable cell growth inhibition against five human cancer cell lines. In the present study isoxazoles **1a** and **1b** were considerably more active than their pyrazole analogs (**12a** and **12b**, [40]) in the sea urchin embryo assay (ca. 50-fold). Similarly, in NCI60 screen isoxazole **1a** was ca. 15-fold more cytotoxic than pyrazole **12a** [40]. In this context it should be noted that GI_{50} values of 5-aminoisoxazole **1a** against non-small cell lung cancer cell line A549 and prostate cancer cell line PC-3 in NCI60 screen were <10 nM (Table S3, Supplementary data), whereas GI_{50} values reported previously were 4.49 μM and 3.03 μM, respectively [45].

3. Conclusions

A series of both 5-amino- and 3-aminodiarylisoxazoles substituted with polyalkoxyaryl pharmacophores has been synthesized. Starting materials for our synthetic scheme were easily

Table 2
Cell growth inhibition of aminoisoxazoles **1a**–**c**, **1e**, **1i**, and **2i** (GI_{50}, nM) in NCI60 screen (NCI Anti-cancer Screening Program; http://dtp.cancer.gov) with CA4 as a reference compound.

Compound	NSC	NCI60 screen, cell growth inhibition (GI_{50}, nM)[a]		
		Mean[b]	OVCAR-8[c]	NCI/ADR-RES[d]
CA4	613729	7.68	1.00	1.00
1a	758029	19.0	13.9	<10
1b	758031	55.0	58.5	19.1
1c	760955	33.0	21.5	<10
1e	758030	389.0	448.0	126.0
1i	767978	2570	3620	563.0
2i	763186	2951	4260	684.0

[a] GI_{50}: Concentration required for 50% cell growth inhibition.
[b] Mean GI_{50} values for 60 human cancer cell lines.
[c] OVCAR-8: ovarian cancer cell line 8.
[d] NCI/ADR-RES: P-glycoprotein-overexpressing multi-drug resistant cell line derived from OVCAR-8.

available from plant extracts. It was found that the condensation of diarylcyanoketones with hydroxylamine hydrochlorides described earlier [45] yielded a mixture of regioisomers. We explored a selective synthesis of 3-amino-4,5-diarylisoxazoles based on the *N*,*O*-acylation of amidoximes followed by their intramolecular cyclization. The targeted molecules were further tested in our phenotypic sea urchin embryo assay to identify compounds with antimitotic microtubule destabilizing activity. It was shown that 5-amino isoxazoles were ca. 2-fold more potent than the respective 3-aminoisoxazoles. Compounds exhibiting 5-aminoisoxazole bridge were found to be considerably more active than their corresponding pyrazole analogs both in the sea urchin embryo assay and in the NCI60 anticancer drug screen. The most potent 5-amino-3,4-diarylisoxazoles identified in the sea urchin embryo assay (**1a**, **b**, and **c**) featured 3,4,5-trimethoxyphenyl, 3,4-methylenedioxy-5-methoxyphenyl, or 3,4-ethylenedioxy-5-methoxyphenyl pharmacophores as ring A and *p*-methoxyphenyl substituent as ring B. These compounds also showed strong in vitro cytotoxicity against human cancer cell lines, including multi-drug resistant cells.

4. Experimental protocols

4.1. Chemistry and chemical methods

NMR spectra were collected on a Bruker DR-500 instrument [working frequencies of 500.13 MHz (^{1}H) and 125.76 MHz (^{13}C)]. Mass spectra were obtained on a Finnigan MAT/INCOS 50 instrument (70 eV) using direct probe injection. Elemental analysis was accomplished with the automated Perkin–Elmer 2400 CHN microanalyzer. The compound purity has been determined by NMR, HPLC, and elemental analyses. Purity of compounds **1a**–**e**, **1h**–**k**, **1m**, **1n**, **1″a**, **1″b**, **1″d**, **2c**, **2h**–**k**, **2′a**–**h**, **2′n**, **2′o**, **2″a**, **2″f**, **4h**, **4l**, **5a**–**f**, **5h**, **5t** was determined to be ≥ 95%.

Polymethoxyphenyl aldehydes, acids, acetonitriles [42], and polymethoxydiarylcyanketones [40] were synthesized from plant polyalkoxyallylbenzenes extracted from dill and parsley as described earlier [41,42].

4.1.1. General procedure for synthesis of mixtures of 5-aminoisoxazoles ***1a–e, h, i–k, m, n*** *and 3-aminoisoxazoles* ***2c, h, i–k***

A mixture of ketonitrile (1.2 mmol), $NH_2OH{\cdot}2HCl$ (2.4 mmol) and $NaOAc{\cdot}3H_2O$ (2.4 mmol) in 5 mL of EtOH was refluxed for 5 h, concentrated *in vacuo*, extracted with 50 mL of CH_2Cl_2. The resulting extract washed with 2 × 50 mL of water, dried over anhydrous Na_2SO_4 and separated by column chromatography (SiO_2, EtOAc/petroleum ether, 1:3–1:5). The yield of targeted mixtures was 25–60%, with the ratio of the isolated isomers 1:1–1:1.5.

*4.1.1.1. 3-(3,4,5-Trimethoxyphenyl)-4-(4-methoxyphenyl)-5-isoxazolamine (**1a**).* White solid; 13% yield; mp 165–167 °C (lit. [45] 157–159 °C); ^{1}H NMR ($CDCl_3$): δ 3.67 (s, 6H, 2× OCH_3-3′,5′), 3.82 (s, 3H, OCH_3-4′), 3.85 (s, 3H, OCH_3-4″), 4.48 (s, 2H, NH_2-5), 6.71 (s, 2H, H-2′,6′), 6.93 (d, *J* = 8.7 Hz, 2H, H-3″,5″), 7.17 (d, *J* = 8.7 Hz, 2H, H-2″,6″); ^{13}C NMR (DMSO-d_6): δ 55.52 (CH_3, OCH_3-4″), 55.95 (2CH_3, OCH_3-3′,5′), 60.43 (CH_3, OCH_3-4′), 91.12 (C, C-4), 105.63 (2CH, C-2′,6′), 114.44 (2CH, C-3″,5″), 123.30 (C, C-1″), 125.66 (C, C-1′), 131.57 (2CH, C-2″,6″), 138.43 (C, C-4′), 153.00 (2C, C-3′,5′), 158.38 (C, C-4″), 160.93 (C, C-3), 167.63 (C, C-5); EIMS *m*/*z* 356 $[M]^+$ (27), 341 (13), 195 (100), 194 (39), 135 (67), 134 (50), 92 (15), 77 (34), 44 (37); Anal. Calcd for $C_{19}H_{20}N_2O_5$: C 64.04; H 5.66; N 7.86. Found: C 64.23; H 5.76; N 7.67.

*4.1.1.2. 3-(7-Methoxy-1,3-benzodioxol-5-yl)-4-(4-methoxyphenyl)-5-isoxazolamine (**1b**).* White solid; 32% yield; mp 128–130 °C; ^{1}H NMR ($CDCl_3$): δ 3.73 (s, 3H, OCH_3-7′), 3.82 (s, 3H, OCH_3-4″), 4.48 (s, 2H, NH_2-5), 5.96 (s, 2H, OCH_2O), 6.62 (d, *J* = 2.4 Hz, 1H, H-6′), 6.72 (d, *J* = 2.4 Hz, 1H, H-4′), 6.92 (d, *J* = 8.8 Hz, 2H, H-3″,5″), 7.14 (d, *J* = 8.8 Hz, 2H, H-2″,6″); ^{13}C NMR (DMSO-d_6): δ 55.00 (CH_3, OCH_3-4″), 55.90 (CH_3, OCH_3-7′), 90.61 (C, C-4), 101.46 (CH_2, OCH_2O), 101.63 (CH, C-4′), 107.86 (CH, C-6′), 114.02 (2CH, C-3″,5″), 122.74 (C, C-1″), 123.98 (C, C-5′), 130.85 (2CH, C-2″,6″), 135.40 (C, C-1′), 142.90 (C, C-7′), 148.21 (C, C-3′), 157.81 (C, C-4″), 160.41 (C, C-3), 167.14 (C, C-5); EIMS *m*/*z* 340 $[M]^+$ (43), 312 (15), 179 (100), 178 (74), 135 (89), 134 (79), 119 (31), 77 (54), 76 (47), 44 (58); Anal. Calcd for $C_{18}H_{16}N_2O_5$: C 63.52; H 4.74; N 8.23. Found: C 63.64; H 4.76; N 8.12.

*4.1.1.3. 3-(2,3-Dihydro-8-methoxy-1,4-benzodioxin-6-yl)-4-(4-methoxyphenyl)-5-isoxazolamine (**1c**).* White solid; 20% yield; mp 164–167 °C (HPLC); ^{1}H NMR (DMSO-d_6): δ 3.56 (s, 3H, OCH_3-8′), 3.75 (s, 3H, OCH_3-4″), 4.20 (m, 4H, OCH_2CH_2O), 6.42 (d, *J* = 1.9 Hz, 1H, H-7′), 6.51 (d, *J* = 1.9 Hz, 1H, H-5′), 6.60 (s, 2H, NH_2-5), 6.93 (d, *J* = 8.7 Hz, 2H, H-3″,5″), 7.08 (d, *J* = 8.7 Hz, 2H, H-2″,6″); ^{13}C NMR (DMSO-d_6): δ 55.30 (CH_3, OCH_3-4″), 55.77 (CH_3, OCH_3-8′), 64.19 (2CH_2, OCH_2CH_2O), 90.31 (C, C-4), 104.12 (CH, C-7′), 108.70 (CH, C-5′), 114.66 (2CH, C-3″,5″), 120.56 (C, C-1″), 121.88 (CH, C-6′), 131.80(CH, C-4′) (2CH, C-2″,6″), 134.10 (C, C-1′), 141.92 (CH, C-4′), 148.77 (CH, C-8′), 159.91 (C, C-4″), 161.73 (C, C-3), 167.54(C, C-5); EIMS *m*/*z* 354 $[M]^+$ (5), 193 (11), 192 (8), 149 (15), 135 (9), 134 (6), 119 (7), 111 (17), 109 (14), 107 (6), 97 (30), 57 (100), 43 (84); Anal. Calcd for $C_{19}H_{18}N_2O_5$: C 64.40; H 5.12; N 7.91. Found: C 64.34; H 5.07; N 7.81.

*4.1.1.4. 3-(4,7-Dimethoxy-1,3-benzodioxol-5-yl)-4-(4-methoxyphenyl)-5-isoxazolamine (**1d**).* White solid; 25% yield; mp 162–164 °C; ^{1}H NMR ($CDCl_3$): δ 3.41 (s, 3H, OCH_3-4′), 3.78 (s, 3H, OCH_3-7′), 3.82 (s, 3H, OCH_3-4″), 4.53 (s, 2H, NH_2-5), 5.98 (s, 2H, OCH_2O), 6.59 (s, 1H, H-6′), 6.86 (d, *J* = 8.7 Hz, 2H, H-3″,5″), 7.07 (d, *J* = 8.7 Hz, 2H, H-2″,6″); ^{13}C NMR (DMSO-d_6): δ 54.92 (CH_3, OCH_3-4″), 56.42 (CH_3, OCH_3-7′), 59.15 (CH_3, OCH_3-4′), 92.42 (C, C-4), 101.84 (CH_2, OCH_2O), 109.13 (CH, C-6′), 113.80 (2CH, C-3″,5″), 116.23 (C, C-5′), 123.56 (C, C-1″), 128.70 (2CH, C-2″,6″), 135.71 (CH, C-4′), 137.19 (C, C-1′), 138.38 (C, C-3′), 138.59 (C, C-7′), 157.21 (C, C-4″), 159.72 (C, C-3), 165.83 (C, C-5); EIMS *m*/*z* 370 $[M]^+$ (15), 209 (11), 208 (9), 163 (11), 162 (16), 135 (99), 134 (100), 119 (41), 107 (24), 106 (31), 93 (42), 92 (31), 91 (33), 77 (75); Anal. Calcd for $C_{19}H_{18}N_2O_6$: C 61.62; H 4.90; N 7.56. Found: C 61.74; H 4.96; N 7.42.

*4.1.1.5. 3-(6,7-Dimethoxy-1,3-benzodioxol-5-yl)-4-(4-methoxyphenyl)-5-isoxazolamine (**1e**).* Resin; 12% yield; ^{1}H NMR (DMSO-d_6): δ 3.36 (s, 3H, OCH_3-6′), 3.70 (s, 3H, OCH_3-7′), 3.89 (s,

3H, OCH_3-4″), 6.03 (s, 2H, OCH_2O), 6.46 (s, 1H, H-4′), 6.68 (s, 2H, NH_2-5), 6.83 (d, J = 8.8 Hz, 2H, H-3″,5″), 6.99 (d, J = 8.8 Hz, 2H, H-2″,6″); ^{1}H NMR ($CDCl_3$): δ 3.53 (s, 3H, OCH_3-6′), 3.77 (s, 3H, OCH_3-7′), 3.97 (s, 3H, OCH_3-4″), 4.53 (s, 2H, NH_2-5), 5.95 (s, 2H, OCH_2O), 6.47 (s, 1H, H-4′), 6.83 (d, J = 8.8 Hz, 2H, H-3″,5″), 7.07 (d, J = 8.8 Hz, 2H, H-2″,6″); ^{13}C NMR (DMSO-d_6): δ 54.88 (CH_3, OCH_3-4″), 59.75 (CH_3, OCH_3-7′), 60.94 (CH_3, OCH_3-6′), 92.28 (C, C-4), 101.71 (CH_2, OCH_2O), 103.23 (CH, C-4′), 113.80 (2CH, C-3″,5″), 116.79 (C, C-5′), 123.30 (C, C-1″), 128.74 (2CH, C-2″,6″), 137.08 (C, C-7′), 138.37 (C, C-1′), 144.04(C, C-3′), 144.88 (CH, C-6′), 157.16 (C, C-4″), 159.62 (C, C-3), 165.87 (C, C-5); EIMS m/z 370 $[M]^+$ (100), 339 (6), 327 (18), 312 (21), 311 (60), 209 (38), 208 (29), 193 (29), 192 (30), 179 (11), 135 (94), 134 (77), 107 (22), 91 (70), 77 (64), 44 (75); Anal. Calcd for $C_{19}H_{18}N_2O_6$: C 61.62; H 4.90; N 7.56. Found: C 61.79; H 4.98; N 7.39.

*4.1.1.6. 3-(4-Methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-5-isoxazolamine (**1h**).* White solid; 18% yield; mp 156–158 °C (lit. [45] 146–148 °C); ^{1}H NMR (DMSO-d_6): δ 3.63 (s, 6H, 2× OCH_3-3″,5″), 3.65 (s, 3H, OCH_3-4″), 3.76 (s, 3H, OCH_3-4′), 6.35 (s, 2H, H-2″,6″), 6.78 (s, 2H, NH_2-5), 6.95 (d, J = 8.7 Hz, 2H, H-3′,5′), 7.30 (d, J = 8.7 Hz, 2H, H-2′,6′); ^{13}C NMR (DMSO-d_6): δ 55.21 (CH_3, OCH_3-4′), 55.59 (2CH_3, OCH_3-3″,5″), 60.00 (CH_3, OCH_3-4″), 91.12 (C, C-4), 106.46 (2CH, C-2″,6″), 113.84 (2CH, C-3′,5′), 122.19 (C, C-1′), 126.42 (C, C-1″), 129.56 (2CH, C-2′,6′), 135.78 (C, C-4″), 152.82 (2C, C-3″,5″), 159.92 (C, C-4′), 160.81 (C, C-3), 166,99 (C, C-5); EIMS m/z 356 $[M]^+$ (13), 341 (2), 328 (2), 313 (2), 297 (2), 208 (2), 195 (5), 194 (6), 193 (7), 149 (8), 135 (100), 134 (48), 121 (7), 92 (5), 77 (9); Anal. Calcd for $C_{19}H_{20}N_2O_5$: C 64.04; H 5.66; N 7.86. Found: C 64.19; H 5.71; N 7.74.

*4.1.1.7. 4-(7-Methoxy-1,3-benzodioxol-5-yl)-3-(4-methoxyphenyl)-5-isoxazolamine (**1i**).* White solid; 12% yield; mp 162–164 °C; ^{1}H NMR (DMSO-d_6): δ 3.71 (s, 3H, OCH_3-7″), 3.76 (s, 3H, OCH_3-4′), 5.97 (s, 2H, OCH_2O), 6.28 (d, J = 1.5 Hz, 1H, H-6″), 6.35 (d, J = 1.5 Hz, 1H, H-4″), 6.71 (s, 2H, NH_2-5), 6.94 (d, J = 8.8 Hz, 2H, H-3′,5′), 7.28 (d, J = 8.8 Hz, 2H, H-2′,6′); ^{13}C NMR (DMSO-d_6): δ 55.18 (CH_3, OCH_3-4′), 56.00 (CH_3, OCH_3-7″), 90.94 (C, C-4), 101.15 (CH_2, OCH_2O), 103.39 (CH, C-4″), 109.03 (CH, C-6″), 113.91 (2CH, C-3′,5′), 122.14 (C, C-1′), 125.09 (C, C-5″), 129.38 (2CH, C-2′,6′), 133.37 (C, C-1″), 143.25 (C, C-7″), 148.41 (C, C-3″), 159.91 (C, C-4′), 160.70 (C, C-3), 167.12 (C, C-5); EIMS m/z 340 $[M]^+$ (57), 339 (25), 325 (8), 312 (10), 309 (1), 192 (32), 179 (16), 178 (21), 135 (100), 134 (61), 92 (10), 77 (18), 43 (43); Anal. Calcd for $C_{18}H_{16}N_2O_5$: C 63.52; H 4.74; N 8.23. Found: C 63.58; H 4.77; N 8.14.

*4.1.1.8. 4-(4,7-Dimethoxy-1,3-benzodioxol-5-yl)-3-(4-methoxyphenyl)-5-isoxazolamine (**1j**).* White solid; 8% yield; mp 145–147 °C; ^{1}H NMR (DMSO-d_6): δ 3.46 (s, 3H, OCH_3-4″), 3.70 (s, 3H, OCH_3-7″), 3.74 (s, 3H, OCH_3-4′), 6.02 (s, 2H, OCH_2O), 6.33 (s, 1H, H-6″), 6.46 (s, 2H, NH_2-5), 6.91 (d, J = 8.8 Hz, 2H, H-3′,5′), 7.28 (d, J = 8.8 Hz, 2H, H-2′,6′); EIMS m/z 370 $[M]^+$ (51), 339 (36), 327 (5), 222 (19), 207 (22), 193 (7), 170 (12), 163 (17), 149 (23), 135 (100), 134 (41), 107 (15), 92 (28), 77 (44), 44 (49); Anal. Calcd for $C_{19}H_{18}N_2O_6$: C 61.62; H 4.90; N 7.56. Found: C 61.74; H 4.95; N 7.48.

*4.1.1.9. 4-(6,7-Dimethoxy-1,3-benzodioxol-5-yl)-3-(4-methoxyphenyl)-5-isoxazolamine (**1k**).* White solid; 12% yield; mp 158–161 °C; ^{1}H NMR (DMSO-d_6): δ 3.42 (s, 3H, OCH_3-6″), 3.74 (s, 3H, OCH_3-7″), 3.92 (s, 3H, OCH_3-4′), 5.99 (s, 2H, OCH_2O), 6.28 (s, 1H, H-4″), 6.42 (s, 2H, NH_2-5), 6.91 (d, J = 8.9 Hz, 2H, H-3′,5′), 7.30 (d, J = 8.9 Hz, 2H, H-2′,6′); EIMS m/z 370 $[M]^+$ (100), 355 (29), 339 (88), 327 (11), 235 (6), 207 (13), 135 (19), 43 (29); Anal. Calcd for $C_{19}H_{18}N_2O_6$: C 61.62; H 4.90; N 7.56. Found: C 61.77; H 4.96; N 7.45.

*4.1.1.10. 4-(3,4-Dimethoxyphenyl)-3-phenyl-5-isoxazolamine (**1m**).* White solid; 55% yield; mp 147–149 °C; ^{1}H NMR (DMSO-d_6): δ 3.61 (s, 3H, OCH_3-3″), 3.74 (s, 3H, OCH_3-4″), 6.62 (dd, J = 8.2, 2.0 Hz, 1H, H-6″), 6.65 (d, J = 2.0 Hz, 1H, H-2″), 6.66 (s, 2H, NH_2-5), 6.89 (d, J = 8.2 Hz, 1H, H-5″), 7.37 (m, 5H, C_6H_5); EIMS m/z 296 $[M]^+$ (21), 281 (4), 268 (15), 253 (8), 250 (1), 178 (8), 165 (22), 164 (29), 163 (21), 148 (9), 135 (5), 119 (25), 105 (100), 104 (86), 89 (10), 77 (55); Anal. Calcd for $C_{17}H_{16}N_2O_3$: C 68.91; H 5.44. Found: C 68.87; H 5.33.

*4.1.1.11. 4-(4-Chlorophenyl)-3-(4-methoxyphenyl)-5-isoxazolamine (**1n**).* Yield 65% (mixture of 3-amino- and 5-aminoizoxazoles = 6:1 according to ^{1}H NMR analysis). **1n**: white solid; mp 148–150 °C; R_f = 0.40; ^{1}H NMR (DMSO-d_6): δ 3.76 (s, 3H, OCH_3-4′), 6.87 (s, 2H, NH_2-5), 6.94 (d, J = 8.8 Hz, 2H, H-3′,5′), 7.11 (d, J = 8.5 Hz, 2H, H-3″,5″), 7.23 (d, J = 8.8 Hz, 2H, H-2′,6′), 7.36 (d, J = 8.5 Hz, 2H, H-2″,6″); EIMS m/z 302 $[M+2]^+$ (17), 300 $[M]^+$ (50), 287 (5), 285 (18), 272 (5), 257 (10), 242 (16), 165 (10), 153 (11), 152 (21), 151 (35), 150 (17), 149 (87), 148 (41), 139 (19), 138 (21), 135 (100), 134 (82), 125 (11), 123 (38), 113 (7), 111 (19), 108 (21), 92 (24), 89 (17), 77 (34); Anal. Calcd for $C_{16}H_{13}ClN_2O_2$: C 63.90; H 4.36; N 9.31. Found: C 63.82; H 4.29; N 9.39. Corresponding 3-aminoizoxazole was not separated as pure compound.

*4.1.1.12. 3-Amino-4-(4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-3-isoxazolamin (**2a**).* This was found in evaporated reaction mixture by ^{1}H NMR analysis but was not separated.

*4.1.1.13. 5-(2,3-Dihydro-8-methoxy-1,4-benzodioxin-6-yl)-4-(4-methoxyphenyl)-3-isoxazolamine (**2c**).* White solid; 15% yield; mp 189–191 °C (HPLC); ^{1}H NMR (DMSO-d_6): δ 3.60 (s, 3H, OCH_3-8″), 3.80 (s, 3H, OCH_3-4′), 4.20 (m, 4H, OCH_2CH_2O), 5.27 (s, 2H, NH_2-3), 6.49 (d, J = 1.9 Hz, 1H, H-7″), 6.63 (d, J = 1.9 Hz, 1H, H-5″), 7.04 (d, J = 8.7 Hz, 2H, H-3′,5′), 7.25 (d, J = 8.7 Hz, 2H, H-2′,6′); EIMS m/z 354 $[M]^+$ (84), 311 (5), 283 (15), 255 (12), 193 (66), 192 (49), 161 (80), 149 (14), 135 (14), 134 (76), 119 (21), 111 (14), 109 (17), 107 (16), 97 (23), 57 (100), 43 (99); Anal. Calcd for $C_{19}H_{18}N_2O_5$: C 64.40; H 5.12; N 7.91. Found: C 64.26; H 5.04; N 7.98.

*4.1.1.14. 5-(4-Methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-3-isoxazolamine (**2h**).* White solid; 11% yield; mp 156–158 °C; ^{1}H NMR (DMSO-d_6): δ 3.71 (s, 3H, OCH_3-4′), 3.72 (s, 6H, 2× OCH_3-3′,5′), 3.76 (s, 3H, OCH_3-4″), 5.38 (s, 2H, NH_2-3), 6.59 (s, 2H, H-2′,6′), 6.97 (d, J = 8.7 Hz, 2H, H-3″,5″), 7.43 (d, J = 8.7 Hz, 2H, H-2″,6″); ^{13}C NMR (DMSO-d_6): δ 55.08 (CH_3, OCH_3-4″), 55.76 (2CH_3, OCH_3-3′,5′), 59.87 (CH_3, OCH_3-4′), 106.57 (C, C-4), 106.91 (2CH, C-2′,6′), 114.12 (2CH, C-3″,5″), 120.31 (C, C-1″), 124.97 (C, C-1′), 127.80 (2CH, C-2″,6″), 136.98 (C, C-4′), 153.21 (2C, C-3′,5′), 160.12 (C, C-4″), 162.04 (C, C-5), 162.94 (C, C-3); EIMS m/z 356 $[M]^+$ (49), 341 (14), 313 (6), 270 (2), 255 (3), 254 (4), 253 (2), 238 (4), 221 (19), 194 (20), 193 (11), 162 (6), 149 (15), 135 (100), 134 (49), 119 (5), 107 (8), 92 (13), 77 (23); Anal. Calcd for $C_{19}H_{20}N_2O_5$: C 64.04; H 5.66; N 7.86. Found: C 64.12; H 5.69; N 7.79.

*4.1.1.15. 4-(7-Methoxy-1,3-benzodioxol-5-yl)-5-(4-methoxyphenyl)-3-isoxazolamine (**2i**).* White solid; 22% yield; mp 178–181 °C; ^{1}H NMR (DMSO-d_6): δ 3.77 (s, 3H, OCH_3-7′), 3.79 (s, 3H, OCH_3-4″), 5.35 (s, 2H, NH_2-3), 6.05 (s, 2H, OCH_2O), 6.50 (d, J = 1.4 Hz, 1H, H-4′), 6.57 (d, J = 1.4 Hz, 1H, H-6′), 6.97 (d, J = 8.9 Hz, 2H, H-3″,5″), 7.41 (d, J = 8.9 Hz, 2H, H-2″,6″); ^{13}C NMR (DMSO-d_6): δ 55.15 (CH_3, OCH_3-4″), 56.12 (CH_3, OCH_3-7′), 101.33 (CH_2, OCH_2O), 103.66 (CH, C-4′), 106.37 (C, C-4), 109.41 (CH, C-6′), 114.16 (2CH, C-3″,5″), 120.28 (C, C-1″), 123.47 (C, C-5′), 127.72 (2CH, C-2″,6″), 134.53 (C, C-1′), 143.57 (C, C-7′), 148.72 (C, C-3′), 160.08 (C, C-4″), 162.03 (C, C-5), 162.99 (C, C-3); EIMS m/z 340 $[M]^+$ (45), 325 (6), 312 (10), 205 (17), 178 (21), 135 (100), 134 (49), 92 (20), 77 (32), 43 (37); Anal. Calcd for $C_{18}H_{16}N_2O_5$: C 63.52; H 4.74; N 8.23. Found: C 63.60; H 4.75; N 8.17.

*4.1.1.16. 4-(4,7-Dimethoxy-1,3-benzodioxol-5-yl)-5-(4-methoxyphenyl)-3-isoxazolamine (**2j**).* White solid; 17% yield; mp 118–120 °C; ^{1}H NMR (DMSO-d_6): δ 3.61 (s, 3H, OCH_3-4′), 3.75 (s, 3H, OCH_3-7′), 3.76 (s, 3H, OCH_3-4″), 5.21 (s, 2H, NH_2-3), 6.08 (s, 2H, OCH_2O), 6.47 (s, 1H, H-6′), 6.96 (d, J = 8.9 Hz, 2H, H-3″,5″), 7.38 (d, J = 8.9 Hz, 2H, H-2″,6″); EIMS m/z 370 $[M]^+$ (69), 355 (3), 236 (6), 235 (41), 208 (14), 193 (20), 136 (11), 135 (100), 134 (39), 107 (17), 92 (31), 77 (48), 43 (26); Anal. Calcd for $C_{19}H_{18}N_2O_6$: C 61.62; H 4.90; N 7.56. Found: C 61.68; H 4.93; N 7.51.

*4.1.1.17. 4-(6,7-Dimethoxy-1,3-benzodioxol-5-yl)-5-(4-methoxyphenyl)-3-isoxazolamine (**2k**).* White solid; 55% yield; mp 172–175 °C; ^{1}H NMR (DMSO-d_6): δ 3.48 (s, 3H, OCH_3-6′), 3.76 (s, 3H, OCH_3-7′), 3.96 (s, 3H, OCH_3-4″), 5.29 (s, 2H, NH_2-3), 6.06 (s, 2H, OCH_2O), 6.46 (s, 1H, H-6′), 6.96 (d, J = 8.9 Hz, 2H, H-3″,5″), 7.38 (d, J = 8.9 Hz, 2H, H-2″,6″); EIMS m/z 370 $[M]^+$ (100), 355 (3), 339 (4), 338 (6), 327 (2), 312 (11), 235 (35), 193 (17), 135 (67), 134 (20), 107 (11), 92 (19), 77 (33), 44 (18); Anal. Calcd for $C_{19}H_{18}N_2O_6$: C 61.62; H 4.90; N 7.56. Found: C 61.70; H 4.93; N 7.45.

*4.1.2. General procedure for synthesis of diacyl derivatives **1″a**,**b**,**d***

A suspension of 5-aminoisoxazole **1a**,**b**,**d** (1.46 mmol) in 2 mL pyridine was treated with 0.37 g (3.62 mmol) of Ac_2O. The reaction mixture was stirred for 24 h, poured into EtOAc (30 mL), washed with 5% aqueous HCl (2 × 15 mL), water (2 × 20 mL), concentrated, and purified by column chromatography (EtOAc/petroleum ether, 1:6, R_f = 0.5). Intermediate monoacyl derivatives **1′a**,**b**,**d** were not separated.

*4.1.2.1. N-Acetyl-N-[4-(4-methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)-5-isoxazolyl]acetamide (**1″a**).* Yellow solid; 45% yield; mp 125–127 °C (lit. [45]. 119–121 °C); ^{1}H NMR ($CDCl_3$): δ 2.32 (s, 6H, 2× NC(O)CH_3), 3.67 (s, 6H, 2× OCH_3-3′,5′), 3.82 (s, 3H, OCH_3-4′), 3.87 (s, 3H, OCH_3-4″), 6.74 (s, 2H, H-2′,6′), 6.92 (d, J = 8.8 Hz, 2H, H-3″,5″), 7.12 (d, J = 8.8 Hz, 2H, H-2″,6″); EIMS m/z 440 $[M]^+$ (13), 398 (17), 357 (10), 356 (13), 210 (23), 195 (10), 194 (11), 168 (39), 147 (51), 135 (18), 43 (100); Anal. Calcd for $C_{23}H_{24}N_2O_7$: C 62.72; H 5.49; N 6.36. Found: C 62.84; H 5.54; N 6.29.

*4.1.2.2. N-Acetyl-N-[3-(7-methoxy-1,3-benzodioxol-5-yl)-4-(4-methoxyphenyl)-5-isoxazolyl]acetamide (**1″b**).* White solid; 65% yield; mp 150–152 °C; ^{1}H NMR ($CDCl_3$): δ 2.31 (s, 6H, 2× NC(O)CH_3), 3.73 (s, 3H, OCH_3-7′), 3.83 (s, 3H, OCH_3-4″), 6.01 (s, 2H, OCH_2O), 6.67 (d, J = 1.5 Hz, 1H, H-6′), 6.73 (d, J = 1.5 Hz, 1H, H-4′), 6.91 (d, J = 8.8 Hz, 2H, H-3″,5″), 7.08 (d, J = 8.8 Hz, 2H, H-2″,6″); EIMS m/z 424 $[M]^+$ (4), 382 (7), 167 (12), 147 (16), 43 (100); Anal. Calcd for $C_{22}H_{20}N_2O_7$: C 62.26; H 4.75; N 6.60. Found: C 62.34; H 4.78; N 6.46.

*4.1.2.3. N-Acetyl-N-[3-(4,7-dimethoxy-1,3-benzodioxol-5-yl)-4-(4-methoxyphenyl)-5-isoxazolyl]acetamide (**1″d**).* White solid; 14% yield; mp 156–162 °C; ^{1}H NMR ($CDCl_3$): δ 2.32 (s, 6H, 2× NC(O)CH_3), 3.36 (s, 3H, OCH_3-4′), 3.80 (s, 3H, OCH_3-7′), 3.85 (s, 3H, OCH_3-4″), 5.99 (s, 2H, OCH_2O), 6.64 (c, 1H, H-6′), 6.87 (d, J = 8.8 Hz, 2H, H-3″,5″), 7.08 (d, J = 8.8 Hz, 2H, H-2″,6″); EIMS m/z 454 $[M]^+$ (1), 412(16), 371 (6), 209 (10), 197 (34), 182 (11), 162 (10), 147 (10), 135 (18), 91 (19), 43 (100); Anal. Calcd for $C_{23}H_{22}N_2O_8$: C 60.79; H 4.88; N 6.16. Found: C 60.84; H 4.92; N 6.10.

*4.1.3. General procedure for synthesis of amidoximes **3p**,**q**,**s***

The solution of NaOH (2.71 g, 67.93 mmol) in water (20 mL) was added at room temperature to the solution of polymethoxyphenylacetonitrile [40] (5.0 g, 33.96 mmol) and hydroxylamine hydrochloride (4.72 g, 67.93 mmol) in EtOH (100 mL). The reaction mixture was refluxed for 5 h, the solvent was evaporated *in vacuo*, and the residue was washed by water (3 × 30 mL) and dried. Yield 75–80%.

*4.1.3.1. N′-Hydroxy-2-(4-methoxyphenyl)ethanimidamide (**3p**).* White solid; 85% yield; mp 88–91 °C (lit. [60] 108–109 °C); ^{1}H NMR (DMSO-d_6): δ 3.18 (s, 2H, CH_2), 3.71 (s, 3H, OCH_3-4), 5.33 (s, 2H, NH_2), 6.84 (d, J = 8.7 Hz, 2H, H-3,5), 7.18 (d, J = 8.7 Hz, 2H, H-2,6), 8.85 (s, 1H, OH); EIMS m/z 180 $[M]^+$ (54), 163 (51), 147 (71), 132 (35), 121 (100), 107 (18), 104 (15), 91 (23), 77 (35); Anal. Calcd for $C_9H_{12}N_2O_2$: C 59.99; H 6.71; N 15.55. Found: C 59.84; H 6.67; N 15.73.

*4.1.3.2. N′-Hydroxy-2-(3,4,5-trimethoxyphenyl)ethanimidamide (**3q**).* White solid; 75% yield; mp 126–128 °C; ^{1}H NMR (DMSO-d_6): δ 3.18 (s, 2H, CH_2), 3.62 (s, 3H, OCH_3-4), 3.75 (s, 6H, OCH_3-3,5), 5.35 (s, 2H, NH_2), 6.59 (s, 2H, H-2,6), 8.86 (s, 1H, OH); EIMS m/z 240 $[M]^+$ (37), 223 (17), 209 (25), 192 (13), 181 (100), 167 (20), 150 (43), 107 (21), 79 (33), 77 (37); Anal. Calcd for $C_{11}H_{16}N_2O_4$: C 54.99; H 6.71; N 11.66. Found: C 55.11; H 6.74; N 11.57.

*4.1.3.3. N′-Hydroxy-2-(4-nitrophenyl)ethanimidamide (**3s**).* White solid; 88% yield; mp 168–170 °C (lit. [61] 170 °C); ^{1}H NMR (DMSO-d_6): δ 3.42 (s, 2H, CH_2), 5.54 (s, 2H, NH_2), 7.55 (d, J = 8.7 Hz, 2H, H-2,6), 8.17 (d, J = 8.7 Hz, 2H, H-3,5), 9.01 (s, 1H, OH); EIMS m/z 195 $[M]^+$ (50), 178 (12), 161 (6), 156 (7), 154 (7), 136 (11), 132 (100), 121 (100), 104 (20), 90 (30), 89 (49), 78 (30), 77 (21); Anal. Calcd for $C_8H_9N_3O_3$: C 49.23; H 4.65; N 21.53. Found: C 49.08; H 4.59; N 21.73.

*4.1.4. General procedure for synthesis of oxadiazoles **5a**–**f**, **h**, **t***

A solution of amidoxime (5 mmol) and benzoylchloride (7.5 mmol) in dry pyridine (10 mL) was refluxed for 1 h, brought to room temperature, poured into ice cold water (100 mL). The resulting precipitate of **5** was collected, washed with 2× 10 mL of cold water and purified by column chromatography (SiO_2, EtOAc/petroleum ether). Yield 12–56%.

In our hands the same procedure [57] using N′-hydroxy-2-(3,4,5-trimethoxyphenyl)ethanimidamide and *p*-methoxybenzoylchloride furnished 3-aminoisoxazole **4h**, whereas the reaction between *N*′-hydroxy-2-(4-chlorophenyl)ethanimidamide and *p*-methoxybenzoylchloride afforded only 3-aminoisoxazole **4l**.

*4.1.4.1. 4-Methoxy-N-[5-(4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-3-isoxazolyl]benzamide (**4h**).* White solid; 10% yield; mp 205–207 °C; ^{1}H NMR (DMSO-d_6): δ 3.68 (s, 3H, OCH_3-4″), 3.72 (s, 6H, 2× OCH_3-3″,5″), 3.85 (s, 3H, OCH_3-4‴), 3.86 (s, 3H, OCH_3-4′), 7.02 (s, 2H, H-2″,6″), 7.09 (d, J = 8.9 Hz, 2H, H-3‴,5‴), 7.14 (d, J = 8.9 Hz, 2H, H-3′,5′), 8.03 (d, J = 8.9 Hz, 2H, H-2‴,6‴), 8.07 (d, J = 8.9 Hz, 2H, H-2′,6′), 10.38 (s, 1H, NH); EIMS m/z 490 $[M]^+$ (17), 195 (16), 135 (97), 107 (5), 92 (7), 77 (12), 43 (100); Anal. Calcd for $C_{27}H_{26}N_2O_7$: C 66.11; H 5.34; N 5.71. Found: C 66.22; H 5.38; N 5.62.

*4.1.4.2. N-[4-(4-Chlorophenyl)-5-(4-methylphenyl)-3-isoxazolyl]-4-methylbenzamide (**4l**).* White solid; 43% yield; mp 236–237 °C (lit. [57] 233–234 °C); ^{1}H NMR (DMSO-d_6): δ 2.40 (s, 3H, CH_3-4‴), 2.41 (s, 3H, CH_3-4′), 7.37 (d, J = 8.1 Hz, 2H, H-3‴,5‴), 7.40 (d, J = 8.1 Hz, 2H, H-3′,5′), 7.55 (d, J = 8.7 Hz, 2H, H-3″,5″), 7.73 (d, J = 8.7 Hz, 2H, H-2″,6″), 7.95 (d, J = 8.1 Hz, 2H, H-2‴,6‴), 8.00 (d, J = 8.1 Hz, 2H, H-2′,6′), 10.56 (s, 1H, NH); EIMS m/z 404 $[M + 2]^+$ (24), 402 $[M]^+$ (68), 141 (13), 139 (41), 119 (100), 113 (7), 111 (29), 91 (80); Anal. Calcd for $C_{24}H_{19}ClN_2O_2$: C 71.55; H 4.75; N 6.95; Cl 8.80. Found: C 71.69; H 4.81; N 6.87; Cl 8.67.

*4.1.4.3. 3-(4-Methoxybenzyl)-5-(3,4,5-trimethoxyphenyl)-1,2,4-oxadiazole (**5a**).* White solid; 32% yield; mp 116–118 °C; ^{1}H NMR (DMSO-d_6): δ 3.73 (s, 3H, OCH_3-4′), 3.76 (s, 3H, OCH_3-4″), 3.88 (s,

6H, 2× OCH_3-3″,5″), 4.09 (s, 2H, CH_2), 6.90 (d, J = 8.7 Hz, 2H, H-3′,5′), 7.26 (d, J = 8.7 Hz, 2H, H-2′,6′), 7.32 (s, 2H, H-2″,6″); EIMS m/z 356 $[M]^+$ (34), 341 (2), 196 (13), 195 (55), 122 (12), 121 (100), 91 (9), 78 (16), 77 (20); Anal. Calcd for $C_{19}H_{20}N_2O_5$: C 64.04; H 5.66; N 7.86. Found: C 64.17; H 5.70; N 7.76.

*4.1.4.4. 5-(7-Methoxy-1,3-benzodioxol-5-yl)-3-(4-methoxybenzyl)-1,2,4-oxadiazole (**5b**).* White solid; 22% yield; mp 96–98 °C; ^{1}H NMR (DMSO-d_6): δ 3.73 (s, 3H, OCH_3-4′), 3.91 (s, 3H, OCH_3-7″), 4.06 (s, 2H, CH_2), 6.16 (s, 2H, OCH_2O), 6.90 (d, J = 8.7 Hz, 2H, H-3′,5′), 7.25 (d, J = 1.5 Hz, 1H, H-6″), 7.26 (d, J = 8.7 Hz, 2H, H-2′,6′), 7.34 (d, J = 1.5 Hz, 1H, H-4″); EIMS m/z 340 $[M]^+$ (37), 180 (10), 179 (10), 161 (14), 151 (16), 121 (92), 95 (21), 91 (12), 78 (30), 77 (27); Anal. Calcd for $C_{18}H_{16}N_2O_5$: C 63.52; H 4.74; N 8.23. Found: C 63.39; H 4.68; N 8.37.

*4.1.4.5. 5-(8-Methoxy-2,3-dihydro-1,4-benzodioxin-6-yl)-3-[(4-methoxybenzyl)methyl]-1,2,4-oxadiazole (**5c**).* White solid; 18% yield; mp 125–127 °C; ^{1}H NMR (DMSO-d_6): δ 3.73 (s, 3H, OCH_3-4′), 3.85 (s, 3H, OCH_3-8″), 4.06 (s, 2H, CH_2), 4.30 (m, 2H, H-2″), 4.33 (m, 2H, H-3″), 6.90 (d, J = 8.7 Hz, 2H, H-3′,5′), 7.18 (s, 2H, H-5″,7″), 7.26 (d, J = 8.7 Hz, 2H, H-2′,6′); ^{13}C: 30.61 (CH_2), 55.08 (OCH_3-4′), 56.05 (OCH_3-8″), 63.95 (C-2″), 64.27 (C-3″), 103.70 (C-7″), 109.63 (C-5″), 114.04 (C-3′,5′), 115.03 (C-6″), 127.67 (C-1′), 129.99 (C-2′,6′), 137.45 (C-4″a), 144.10 (C-8″a), 149.31 (C-8″), 158.25 (C-4′), 170.16 (C-3), 174.84 (C-5); EIMS m/z 354 $[M]^+$ (57), 194 (11), 193 (100), 165 (10), 121 (98), 107 (11), 91 (10), 78 (21), 77 (21); Anal. Calcd for $C_{19}H_{18}N_2O_5$: C 64.40; H 5.12; N 7.91. Found: C 64.56; H 5.19; N 7.78.

*4.1.4.6. 5-(4,7-Dimethoxy-1,3-benzodioxol-5-yl)-3-(4-methoxybenzyl)-1,2,4-oxadiazole (**5d**).* White solid; 16% yield; mp 95–97 °C; ^{1}H NMR (DMSO-d_6): δ 3.73 (s, 3H, OCH_3-4′), 3.84 (s, 3H, OCH_3-4″), 3.91 (s, 3H, OCH_3-7″), 4.07 (s, 2H, CH_2), 6.18 (s, 2H, OCH_2O), 6.90 (d, J = 8.7 Hz, 2H, H-3′,5′), 7.18 (s, 1H, H-6″), 7.26 (d, J = 8.7 Hz, 2H, H-2′,6′); EIMS m/z 370 $[M]^+$ (24), 209 (40), 207 (48), 195 (6), 194 (10), 179 (17), 163 (6), 147 (13), 135 (15), 121 (100), 91 (13), 78 (27), 77 (29); Anal. Calcd for $C_{19}H_{18}N_2O_6$: C 61.62; H 4.90; N 7.56. Found: C 61.80; H 4.97; N 7.41.

*4.1.4.7. 5-(6,7-Dimethoxy-1,3-benzodioxol-5-yl)-3-(4-methoxybenzyl)-1,2,4-oxadiazole (**5e**).* White solid; 21% yield; mp 102–104 °C; ^{1}H NMR (DMSO-d_6): δ 3.73 (s, 3H, OCH_3-4′), 3.79 (s, 3H, OCH_3-7″), 3.98 (s, 3H, OCH_3-6″), 4.06 (s, 2H, CH_2), 6.14 (s, 2H, OCH_2O), 6.90 (d, J = 8.7 Hz, 2H, H-3′,5′), 7.10 (s, 1H, H-4″), 7.27 (d, J = 8.7 Hz, 2H, H-2′,6′); EIMS m/z 370 $[M]^+$ (18), 353 (3), 341 (2), 209 (27), 207 (33), 206 (24), 193 (5), 179 (8), 166 (6), 161 (5), 147 (10), 135 (16), 121 (100), 91 (13), 78 (24), 77 (27); Anal. Calcd for $C_{19}H_{18}N_2O_6$: C 61.62; H 4.90; N 7.56. Found: C 61.78; H 4.93; N 7.48.

*4.1.4.8. 5-(3-Methoxyphenyl)-3-(4-methoxybenzyl)-1,2,4-oxadiazole (**5f**).* White solid; 22% yield; mp 52–54 °C; ^{1}H NMR (DMSO-d_6): δ 3.73 (s, 3H, OCH_3-4′), 3.85 (s, 3H, OCH_3-3″), 4.10 (s, 2H, CH_2), 6.90 (d, J = 8.7 Hz, 2H, H-3′,5′), 7.26 (dd, J = 7.8, 2.7 Hz, 1H, H-4″), 7.27 (d, J = 8.7 Hz, 2H, H-2′,6′), 7.53 (t, J = 7.8 Hz, 1H, H-5″), 7.54 (dd, J = 2.7, 2.5 Hz, 1H, H-2″), 7.66 (dd, J = 7.8, 2.5 Hz, 1H, H-6″); EIMS m/z 296 $[M]^+$ (63), 281 (1), 162 (13), 161 (79), 146 (7), 137 (11), 135 (100), 133 (29), 121 (65), 107 (47), 92 (37), 78 (39), 77 (82); Anal. Calcd for $C_{17}H_{16}N_2O_3$: C 68.91; H 5.44; N 9.45. Found: C 68.84; H 5.40; N 9.53.

*4.1.4.9. 5-(4-Methoxyphenyl)-3-[(3,4,5-trimethoxybenzyl)methyl]-1,2,4-oxadiazole (**5h**).* White solid; 12% yield; mp 86–88 °C; ^{1}H NMR (DMSO-d_6): δ 3.63 (s, 3H, OCH_3-4′), 3.76 (s, 6H, 2× OCH_3-3′,5′), 3.86 (s, 3H, OCH_3-4″), 4.06 (s, 2H, CH_2), 6.67 (s, 2H, H-2′,6′), 7.15 (d, J = 8.9 Hz, 2H, H-3″,5″), 8.03 (d, J = 8.9 Hz, 2H, H-2″,6″); ^{13}C: 31.67 (CH_2), 55.64 (OCH_3-4″), 65.89 (OCH_3-3′,5′), 60.00 (OCH_3-4′), 106.36 (C-2′,6′), 114.98 (C-3″,5″), 115.84 (C-6″), 129.83 (C-2″,6″), 131.39 (C-1′), 136.46 (C-4′), 152.93 (C-3′,5′), 163.03 (C-4′), 169.76 (C-3), 174.92 (C-5); EIMS m/z 356 $[M]^+$ (62), 341 (24), 181 (22), 165 (7), 149 (7), 148 (8), 147 (7), 137 (14), 136 (27), 135 (100), 133 (15), 121 (7), 120 (8), 107 (28), 92 (34), 78 (20), 77 (57); Anal. Calcd for $C_{19}H_{20}N_2O_5$: C 64.04; H 5.66; N 7.86. Found: C 64.16; H 5.70; N 7.77.

*4.1.4.10. 5-(4-Nitrophenyl)-3-(4-methoxybenzyl)-1,2,4-oxadiazole (**5t**).* White solid; 56% yield; mp 122–124 °C; ^{1}H NMR (DMSO-d_6): δ 3.73 (s, 3H, OCH_3-4′), 4.14 (s, 2H, CH_2), 6.91 (d, J = 8.7 Hz, 2H, H-3′,5′), 7.29 (d, J = 8.7 Hz, 2H, H-2′,6′), 8.33 (d, J = 8.9 Hz, 2H, H-2″,6″), 8.42 (d, J = 8.9 Hz, 2H, H-3″, 5″); EIMS m/z 311 $[M]^+$ (88), 161 (100), 150 (17), 146 (9), 137 (10), 134 (30), 121 (83), 118 (7), 116 (6), 104 (32), 92 (17), 91 (20), 90 (21), 78 (35), 77 (43), 76 (42); Anal. Calcd for $C_{16}H_{13}N_3O_4$: C 61.73; H 4.21; N 13.50. Found: C 61.84; H 4.30; N 13.36.

*4.1.5. General procedure for synthesis of mixture of monoacetyl-3-aminoisoxazoles **2′a,f** and diacetyl 3-aminoisoxazoles **2″a,f***

A solution of benzoylamidoxime (1.0 g, 3.18 mmol) and Ac_2O (0.86 g, 10.5 mmol) in pyridine (10 mL) was stirred for 24 h at 60 °C, poured into water (50 mL), extracted with CH_2Cl_2 (2× 20 mL), dried over anhydrous Na_2SO_4, concentrated *in vacuo*, and separated by column chromatography (EtOAc/petroleum ether, 1:4–1:1). First fraction (R_f = 0.8) yielded 30 mg of the diacyl derivative **2″a,f**, second fraction (R_f = 0.2) yielded monoacyl derivative **2′a,f** (25 mg). Attempts to remove the acyl group under a variety of experimental conditions were unsuccessful.

*4.1.6. General procedure for synthesis of benzoylamidoximes **6***

A suspension of benzoic acid (8.8 mmol) in dry MeCN (20 mL) was treated with carbonyl diimidazole (11.5 mmol) at 20 °C. The resulting mixture was stirred for 30 min, treated with amidoxime **3p–s** (8.8 mmol), stirred for additional 8 h, and poured into water (100 mL). The solid residue was filtered and recrystallized from 50% EtOH/water.

*4.1.6.1. 2-(4-Methoxyphenyl)-N′-[(3,4,5-trimethoxybenzoyl)oxy]ethanimidamide (**6a**).* White solid; 82% yield; mp 161–163 °C; ^{1}H NMR (DMSO-d_6): δ 3.36 (s, 2H, CH_2), 3.73 (s, 3H, OCH_3-4), 3.74 (s, 3H, OCH_3-4′), 3.85 (s, 6H, 2× OCH_3-3′,5′), 6.51 (s, 2H, NH_2), 6.89 (d, J = 8.6 Hz, 2H, H-3,5), 7.30 (d, J = 8.6 Hz, 2H, H-2,6), 7.31 (s, 2H, H-2′,6′); EIMS m/z 374 $[M]^+$ (0.2), 196 (9), 195 (100), 122 (6), 121 (28), 78 (7), 77 (10); Anal. Calcd for $C_{19}H_{22}N_2O_6$: C 60.95; H 5.92; N 7.48. Found: C 61.07; H 5.96; N 7.42.

*4.1.6.2. N′-{[(7-Methoxy-1,3-benzodioxol-5-yl)carbonyl]oxy}-2-(4-methoxyphenyl)ethanimidamide (**6b**).* White solid; 70% yield; mp 116–118 °C; ^{1}H NMR (DMSO-d_6): δ 3.34 (s, 2H, CH_2), 3.73 (s, 3H, OCH_3-4), 3.89 (s, 3H, OCH_3-7′), 6.11 (s, 2H, OCH_2O), 6.52 (s, 2H, NH_2), 6.89 (d, J = 8.7 Hz, 2H, H-3,5), 7.29 (d, J = 8.7 Hz, 2H, H-2,6), 7.33 (d, J = 1.5 Hz, 1H, H-6′), 7.39 (d, J = 1.5 Hz, 1H, H-4′); EIMS m/z 358 $[M]^+$ (8), 341 (3), 340 (12), 196 (10), 195 (6), 180 (20), 179 (100), 162 (14), 151 (19), 147 (9), 121 (50), 95 (15), 78 (17), 77 (12); Anal. Calcd for $C_{18}H_{18}N_2O_6$: C 60.33; H 5.06; N 7.82. Found: C 60.39; H 5.08; N 7.69.

*4.1.6.3. N′-{[(8-Methoxy-2,3-dihydro-1,4-benzodioxin-6-yl)carbonyl]oxy}-2-(4-methoxyphenyl)ethanimidamide (**6c**).* White solid; 85% yield; mp 165–167 °C; ^{1}H NMR (DMSO-d_6): δ 3.33 (s, 2H, CH_2), 3.73 (s, 3H, OCH_3-4), 3.82 (s, 3H, OCH_3-8′), 4.26 (m, 2H, H-2′), 4.29 (m, 2H, H-3′), 6.49 (s, 2H, NH_2), 6.88 (d, J = 8.6 Hz, 2H, H-3,5), 7.18 (d, J = 1.8 Hz, 1H, H-7′), 7.29 (d, J = 8.6 Hz, 2H, H-2,6), 7.30 (d, J = 1.8 Hz, 1H, H-5′); EIMS m/z 372 $[M]^+$ (2), 355 (3), 354 (15), 210 (83), 195 (17), 193 (100), 165 (10), 164 (21), 163 (16), 154 (15), 139 (17), 122

(39), 121 (60), 111 (14), 107 (10), 91 (10), 78 (20), 77 (25); Anal. Calcd for $C_{19}H_{20}N_2O_6$: C 61.28; H 5.41; N 7.52. Found: C 61.37; H 5.43; N 7.48.

*4.1.6.4. N′-{[(4,7-Dimethoxy-1,3-benzodioxol-5-yl)carbonyl]oxy}-2-(4-methoxyphenyl)ethanimidamide (**6d**).* White solid; 55% yield; mp 124–126 °C; ^{1}H NMR (DMSO-d_6): δ 3.32 (s, 2H, CH_2), 3.73 (s, 3H, OCH_3-4), 3.82 (s, 3H, OCH_3-4′), 3.83 (s, 3H, OCH_3-7′), 6.11 (s, 2H, OCH_2O), 6.30 (s, 2H, NH_2), 6.89 (d, J = 8.7 Hz, 2H, H-3,5), 7.00 (s, 1H, H-6′), 7.29 (d, J = 8.7 Hz, 2H, H-2,6); EIMS m/z 388 $[M]^+$ (5), 371 (1), 370 (2), 357 (6), 211 (8), 210 (37), 209 (100), 194 (17), 166 (9), 147 (7), 134 (9), 121 (72), 106 (12), 93 (9), 78 (21), 77 (20); Anal. Calcd for $C_{19}H_{20}N_2O_7$: C 58.76; H 5.19; N 7.21. Found: C 58.83; H 5.23; N 7.16.

*4.1.6.5. N′-{[(6,7-Dimethoxy-1,3-benzodioxol-5-yl)carbonyl]oxy}-2-(4-methoxyphenyl)ethanimidamide (**6e**).* White solid; 75% yield; mp 125–127 °C; ^{1}H NMR (DMSO-d_6): δ 3.34 (s, 2H, CH_2), 3.73 (s, 6H, 2× OCH_3-4,7′), 3.93 (s, 3H, OCH_3-6′), 6.08 (s, 2H, OCH_2O), 6.33 (s, 2H, NH_2), 6.89 (d, J = 8.6 Hz, 2H, H-3,5), 7.02 (s, 1H, H-4′), 7.28 (d, J = 8.6 Hz, 2H, H-2,6); EIMS m/z 388 $[M]^+$ (3), 371 (1), 370 (2), 357 (7), 226 (6), 211 (6), 210 (18), 209 (100), 194 (5), 166 (8), 164 (7), 147 (6), 134 (8), 121 (50), 91 (6), 78 (13), 77 (13); Anal. Calcd for $C_{19}H_{20}N_2O_7$: C 58.76; H 5.19; N 7.21. Found: C 58.88; H 5.24; N 7.11.

*4.1.6.6. N′-[(3-Methoxybenzoyl)oxy]-2-(4-methoxyphenyl)ethanimidamide (**6f**).* White solid; 72% yield; mp 107–109 °C; ^{1}H NMR (DMSO-d_6): δ 3.35 (s, 2H, CH_2), 3.73 (s, 3H, OCH_3-4), 3.82 (s, 3H, OCH_3-3′), 6.53 (s, 2H, NH_2), 6.89 (d, J = 8.7 Hz, 2H, H-3,5), 7.20 (dd, J = 8.0, 2.6 Hz, 1H, H-4′), 7.30 (d, J = 8.7 Hz, 2H, H-2,6), 7.41 (t, J = 8.0 Hz, 1H, H-5′), 7.56 (dd, J = 2.6, 1.5 Hz, 1H, H-2′), 7.68 (dd, J = 8.0, 1.5 Hz, 1H, H-6′); EIMS m/z 314 $[M]^+$ (6), 297 (1), 296 (2), 162 (23), 147 (20), 135 (100), 121 (36), 107 (26), 92 (20), 78 (16), 77 (33); Anal. Calcd for $C_{17}H_{18}N_2O_4$: C 64.96; H 5.77; N 8.91. Found: C 65.06; H 5.79; N 8.85.

*4.1.6.7. N′-[(4-Methoxybenzoyl)oxy]-2-(4-methoxyphenyl)ethanimidamide (**6g**).* White solid; 85% yield; mp 142–145 °C; ^{1}H NMR (DMSO-d_6): δ 3.32 (s, 2H, CH_2), 3.73 (s, 3H, OCH_3-4), 3.83 (s, 3H, OCH_3-4′), 6.47 (s, 2H, NH_2), 6.89 (d, J = 8.7 Hz, 2H, H-3,5), 7.01 (d, J = 8.9 Hz, 2H, H-3′,5′), 7.29 (d, J = 8.7 Hz, 2H, H-2,6), 8.05 (d, J = 8.9 Hz, 2H, H-2′, 6′); EIMS m/z 314 $[M]^+$ (1), 296 (1), 162 (9), 136 (10), 135 (100), 121 (22), 107 (7), 92 (11), 78 (10), 77 (22); Anal. Calcd for $C_{17}H_{18}N_2O_4$: C 64.96; H 5.77; N 8.91. Found: C 65.02; H 5.80; N 8.84.

*4.1.6.8. N′-[(4-Methoxybenzoyl)oxy]-2-(3,4,5-trimethoxyphenyl) ethanimidamide (**6h**).* White solid; 75% yield; mp 178–180 °C; ^{1}H NMR (DMSO-d_6): δ 3.35 (s, 2H, CH_2), 3.64 (s, 3H, OCH_3-4), 3.78 (s, 6H, 2× OCH_3-3,5), 3.83 (s, 3H, OCH_3-4′), 6.50 (s, 2H, NH_2), 6.73 (s, 2H, H-2,6), 7.02 (d, J = 8.9 Hz, 2H, H-3′,5′), 8.05 (d, J = 8.9 Hz, 2H, H-2′,6′); EIMS m/z 374 $[M]^+$ (4), 356 (1), 207 (6), 181 (8), 152 (6), 136 (10), 135 (100), 92 (5), 77 (9); Anal. Calcd for $C_{19}H_{22}N_2O_6$: C 60.95; H 5.92; N 7.48. Found: C 61.10; H 5.98; N 7.35.

*4.1.7. General procedure for selective synthesis of 3-acetylaminoisoxazoles **2′a**–**h**, **n**, **o** and 3-diacetylaminoisoxazoles **2″a**,**f***

A solution of benzoylamidoxime (5.3 mmol) in dry pyridine (10 mL) was treated dropwise with acetylchloride (11 mmol) at 10 °C (ice bath), the reaction mixture was stirred at room temperature for 1.5 h, and poured into water (100 mL). The precipitate was collected and purified by column chromatography (SiO_2, EtOAc/petroleum ether = 1:5). During this procedure in case of derivative **6d** besides isoxazol **2d** the intermediate acetylated benzoylamidoxime **7d** was isolated.

*4.1.7.1. N-[N′-{[(4,7-Dimethoxy-1,3-benzodioxol-5-yl)carbonyl]oxy}-2-(4-methoxyphenyl)ethanimidoyl]acetamide (**7d**).* White solid; 16% yield; mp 168–170 °C; ^{1}H NMR (DMSO-d_6): δ 2.24 (s, 3H, $COCH_3$), 3.71 (s, 3H, OCH_3-4), 3.81 (s, 3H, OCH_3-7′), 4.06 (s, 3H, OCH_3-5′), 4.11 (s, 2H, CH_2), 6.17 (s, 2H, OCH_2O), 6.87 (d, J = 8.7 Hz, 2H, H-3,5), 7.17 (s, 1H, H-6′), 7.22 (d, J = 8.7 Hz, 2H, H-2,6), 10.88 (s, 1H, NH); EIMS m/z 430 $[M]^+$ (2), 210 (14), 209 (100), 207 (5), 194 (8), 121 (30), 78 (7), 77 (7), 65 (5); Anal. Calcd for $C_{21}H_{22}N_2O_8$: C 58.60; H 5.15; N 6.51. Found: C 58.78; H 5.21; N 6.46.

*4.1.7.2. N-[4-(4-Methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-3-isoxazolyl]acetamide (**2′a**).* White solid; 38% yield; mp 205–207 °C; ^{1}H NMR (DMSO-d_6): δ 2.10 (s, 3H, NC(O)CH_3), 3.74 (s, 3H, OCH_3-4″), 3.81 (s, 3H, OCH_3-4′), 3.90 (s, 6H, 2× OCH_3-3″,5″), 7.06 (d, J = 8.8 Hz, 2H, H-3′,5′), 7.29 (s, 2H, H-2″,6″), 7.68 (d, J = 8.8 Hz, 2H, H-2′,6′), 9.94 (s, 1H, NH-3); EIMS m/z 398 $[M]^+$ (100), 383 (5), 367 (1), 356 (23), 195 (13), 194 (18), 135 (41), 43 (17); Anal. Calcd for $C_{21}H_{22}N_2O_6$: C 63.31; H 5.57; N 7.03. Found: C 63.47; H 5.65; N 6.91.

*4.1.7.3. N-Acetyl-N-[4-(4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-3-isoxazolyl]acetamide (**2″a**).* White solid; 15% yield; mp 205–207 °C; ^{1}H NMR ($CDCl_3$): δ 2.35 (s, 6H, 2× NC(O)CH_3), 3.83 (s, 3H, OCH_3-4″), 3.93 (s, 6H, 2× OCH_3-3″,5″), 3.97 (s, 3H, OCH_3-4′), 6.99 (d, J = 8.8 Hz, 2H, H-3′,5′), 7.30 (s, 2H, H-2″,6″), 7.53 (d, J = 8.8 Hz, 2H, H-2′,6′); EIMS m/z 440 $[M]^+$ (2), 398 (19), 383 (3), 357 (7), 195 (30), 180 (10), 168 (5), 135 (22), 92 (17), 43 (100); Anal. Calcd for $C_{23}H_{24}N_2O_7$: C 62.72; H 5.49; N 6.36. Found: C 62.79; H 5.52; N 6.31.

*4.1.7.4. N-[5-(7-Methoxy-1,3-benzodioxol-5-yl)-4-(4-methoxyphenyl)-3-isoxazolyl]acetamide (**2′b**).* White solid; 24% yield; mp 195–197 °C; ^{1}H NMR (DMSO-d_6): δ 2.09 (s, 3H, NC(O)CH_3), 3.81 (s, 3H, OCH_3-7″), 3.94 (s, 3H, OCH_3-4′), 6.12 (s, 2H, OCH_2O), 7.05 (d, J = 8.8 Hz, 2H, H-3′,5′), 7.25 (d, J = 1.1 Hz, 1H, H-6″), 7.29 (d, J = 1.1 Hz, 1H, H-4″), 7.67 (d, J = 8.8 Hz, 2H, H-2′,6′), 9.93 (s, 1H, NH-3); EIMS m/z 382 $[M]^+$ (24), 340 (27), 179 (31), 178 (31), 163 (15), 135 (100), 107 (11), 77 (31), 43 (70); Anal. Calcd for $C_{20}H_{18}N_2O_6$: C 62.82; H 4.74; N 7.33. Found: C 62.91; H 4.78; N 7.19.

*4.1.7.5. N-[5-(2,3-Dihydro-8-methoxy-1,4-benzodioxin-6-yl)-4-(4-methoxyphenyl)-3-isoxazolyl]acetamide (**2′c**).* White solid; 35% yield; mp 221–223 °C; ^{1}H NMR (DMSO-d_6): δ 2.08 (s, 3H, NC(O)CH_3), 3.81 (s, 3H, OCH_3-8″), 3.87 (s, 3H, OCH_3-4′), 4.30 (m, 4H, OCH_2CH_2O), 6.63 (d, J = 1.9 Hz, 1H, H-5″), 7.05 (d, J = 8.8 Hz, 2H, H-3′,5′), 7.16 (s, 2H, H-5″,7″), 7.65 (d, J = 8.8 Hz, 2H, H-2′,6′), 9.92 (s, 1H, NH); EIMS m/z 396 $[M]^+$ (18), 354 (20), 193 (34), 192 (47), 136 (16), 135 (100), 107 (14), 92 (19), 77 (30), 43 (70); Anal. Calcd for $C_{21}H_{20}N_2O_6$: C 63.63; H 5.09; N 7.07. Found: C 63.74; H 5.14; N 6.94.

*4.1.7.6. N-[5-(4,7-Dimethoxy-1,3-benzodioxol-5-yl)-4-(4-methoxyphenyl)-3-isoxazolyl]acetamide (**2′d**).* White solid; 12% yield; mp 218–222 °C; ^{1}H NMR (DMSO-d_6): δ 2.10 (s, 3H, NC(O)CH_3), 3.80 (s, 3H, OCH_3-4″), 3.87 (s, 3H, OCH_3-7″), 3.95 (s, 3H, OCH_3-4′), 6.17 (s, 2H, OCH_2O), 7.06 (d, J = 8.8 Hz, 2H, H-3′,5′), 7.12 (s, 1H, H-6″), 7.59 (d, J = 8.8 Hz, 2H, H-2′,6′), 9.94 (s, 1H, NH-3); EIMS m/z 412 $[M]^+$ (45), 370 (27), 209 (48), 208 (61), 135 (100), 107 (91), 77 (16), 43 (34); Anal. Calcd for $C_{21}H_{20}N_2O_7$: C 61.16; H 4.89; N 6.79. Found: C 61.21; H 4.88; N 6.69.

*4.1.7.7. N-[5-(6,7-Dimethoxy-1,3-benzodioxol-5-yl)-4-(4-methoxyphenyl)-3-isoxazolyl]acetamide (**2′e**).* White solid; 18% yield; mp 140–143 °C; ^{1}H NMR (DMSO-d_6): δ 2.09 (s, 3H, NC(O)CH_3), 3.70 (s, 3H, OCH_3-6″), 3.80 (s, 3H, OCH_3-7″), 4.00 (s, 3H, OCH_3-4′), 6.11 (s, 2H, OCH_2O), 7.06 (d, J = 8.7 Hz, 2H, H-3′,5′), 7.09 (s, 1H, H-4″), 7.59 (d, J = 8.7 Hz, 2H, H-2′,6′), 9.93 (s, 1H, NH-3); EIMS m/z

412 $[M]^+$ (26), 370 (13), 209 (23), 208 (36), 193 (11), 163 (12), 135 (100), 107 (11), 92 (14), 77 (24), 43 (46); Anal. Calcd for $C_{21}H_{20}N_2O_7$: C 61.16; H 4.89; N 6.79. Found: C 61.29; H 4.97; N 6.64.

*4.1.7.8. N-[5-(3-Methoxyphenyl)-4-(4-methoxyphenyl)-3-isoxazolyl] acetamide (**2′f**).* White solid; 8% yield; mp 185–187 °C; ^{1}H NMR (DMSO-d_6): δ 2.09 (s, 3H, NC(O)CH_3), 3.81 (s, 3H, OCH_3-3″), 3.86 (s, 3H, OCH_3-4′), 7.07 (d, J = 8.8 Hz, 2H, H-3′,5′), 7.12 (dd, J = 8.0, 2.4 Hz, 1H, H-4″), 7.48 (t, J = 8.0 Hz, 1H, H-5″), 7.52 (t, J = 2.4 Hz, 1H, H-2″), 7.63 (d, J = 8.0 Hz, 1H, H-6″), 7.67 (d, J = 8.0 Hz, 2H, H-2′,6′), 9.99 (s, 1H, NH-3); EIMS m/z 338 $[M]^+$ (99), 297 (18), 296 (100), 163 (17), 136 (17), 135 (94), 107 (9), 92 (11), 77 (15), 43 (26); Anal. Calcd for $C_{19}H_{18}N_2O_4$: C 67.45; H 5.36; N 8.28. Found: C 67.59; H 5.42; N 8.17.

*4.1.7.9. N-Acetyl-N-[5-(3-methoxyphenyl)-4-(4-methoxyphenyl)-3-isoxazolyl]acetamide (**2″f**).* Oil; 8% yield; ^{1}H NMR ($CDCl_3$): δ 2.40 (s, 6H, 2× NC(O)CH_3), 3.85 (s, 3H, OCH_3-4′), 3.89 (s, 3H, OCH_3-3″), 6.98 (d, J = 8.9 Hz, 2H, H-3′,5′), 7.04 (dd, J = 8.0, 2.6 Hz, 1H, H-4″), 7.40 (t, J = 8.0 Hz, 1H, H-5″), 7.54 (d, J = 8.9 Hz, 2H, H-2′,6′), 7.59 (t, J = 2.6 Hz, 1H, H-2″), 7.67 (dd, J = 8.0, 2.6 Hz, 1H, H-6″); EIMS m/z 380 $[M]^+$ (8), 398 (19), 338 (28), 297 (10), 296 (50), 204 (30), 161 (47), 135 (100), 121 (41), 107 (60), 92 (71), 43 (100); Anal. Calcd for $C_{21}H_{20}N_2O_5$: C 66.31; H 5.30; N 7.36. Found: C 62.37; H 5.32; N 7.30.

*4.1.7.10. N-[4,5-Bis(4-methoxyphenyl)-3-isoxazolyl]acetamide (**2′g**).* White solid; 16% yield; mp 201–203 °C; ^{1}H NMR (DMSO-d_6): δ 2.09 (s, 3H, NC(O)CH_3), 3.81 (s, 3H, OCH_3-4′), 3.85 (s, 3H, OCH_3-4″), 7.06 (d, J = 8.8 Hz, 2H, H-3′,5′), 7.11 (d, J = 8.8 Hz, 2H, H-3″,5″), 7.63 (d, J = 8.8 Hz, 2H, H-2′,6′), 7.97 (d, J = 8.8 Hz, 2H, H-2″,6″), 9.92 (s, 1H, NH-3); EIMS m/z 338 $[M]^+$ (45), 297 (18), 296 (50), 163 (10), 162 (6), 136 (12), 135 (100), 134 (34), 107 (6), 92 (12), 77 (14), 43 (28); Anal. Calcd for $C_{19}H_{18}N_2O_4$: C 67.45; H 5.36; N 8.28. Found: C 67.49; H 5.39; N 8.21.

*4.1.7.11. N-[5-(4-Methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-3-isoxazolyl]acetamide (**2′h**).* White solid; 26% yield; mp 196–199 °C; ^{1}H NMR (DMSO-d_6): δ 2.11 (s, 3H, NC(O)CH_3), 3.72 (s, 3H, OCH_3-4′), 3.86 (s, 9H, 3× OCH_3-3′,5′,4″), 6.99 (s, 2H, H-2′,6′), 7.12 (d, J = 8.8 Hz, 2H, H-3″,5″), 8.02 (d, J = 8.8 Hz, 2H, H-2″,6″), 10.03 (s, 1H, NH); EIMS m/z 398 $[M]^+$ (68), 356 (16), 342 (13), 341 (64), 222 (16), 195 (60), 135 (100), 134 (40), 92 (9), 77 (18), 43 (74); Anal. Calcd for $C_{21}H_{22}N_2O_6$: C 63.31; H 5.57; N 7.03. Found: C 63.42; H 5.60; N 6.94.

*4.1.7.12. N-[5-(4-Methoxyphenyl)-4-(4-chlorophenyl)-3-isoxazolyl] acetamide (**2′n**).* White solid; 85% yield; mp 252–254 °C (MeOH); ^{1}H NMR (DMSO-d_6): δ 2.11 (s, 3H, $COCH_3$), 3.85 (s, 3H, OCH_3-4″), 7.12 (d, J = 8.5 Hz, 2H, H-3″,5″), 7.54 (d, J = 8.0 Hz, 2H, H-3′,5′), 7.68 (d, J = 8.0 Hz, 2H, H-2′,6′), 8.00 (d, J = 8.5 Hz, 2H, H-2″,6″), 10.11 (s, 1H, NH); EIMS m/z 344 $[M+2]^+$ (4), 342 $[M]^+$ (12), 302 (7), 300 (22), 167 (6), 141 (13), 139 (40), 135 (31), 134 (100), 113 (9), 111 (29), 90 (5), 77 (5), 43 (58); Anal. Calcd for $C_{18}H_{15}ClN_2O_3$: C 63.07; H 4.41; N 8.17. Found: C 63.15; H 4.37; N 8.28.

*4.1.7.13. N-[5-(4-Methoxyphenyl)-4-(4-nitrophenyl)-3-isoxazolyl] acetamide (**2′o**).* White solid; 25% yield; mp 243–247 °C (MeOH); ^{1}H NMR (DMSO-d_6): δ 2.14 (s, 3H, $COCH_3$), 3.86 (s, 3H, OCH_3-4″), 7.14 (d, J = 8.8 Hz, 2H, H-3″,5″), 7.87 (d, J = 8.9 Hz, 2H, H-2′,6′), 8.05 (d, J = 8.8 Hz, 2H, H-2″,6″), 8.31 (d, J = 8.9 Hz, 2H, H-3′,5′), 10.40 (s, 1H, NH); EIMS m/z 353 $[M]^+$ (14), 311 (32), 178 (3), 150 (13), 135 (38), 134 (100), 119 (5), 117 (4), 104 (22), 92 (10), 90 (6), 77 (7), 76 (18), 43 (69); Anal. Calcd for $C_{18}H_{15}N_3O_5$: C 61.19; H 4.28; N 11.89. Found: C 61.25; H 4.36; N 11.81.

4.2. Biology. Sea urchin embryo assay [58]

Adult sea urchins *Paracentrotus lividus* were collected from the Mediterranean Sea at the Cyprus coast and kept in an aerated seawater tank. Gametes were obtained by intracoelomic injection of 0.5 M KCl. Eggs were washed with filtered seawater and fertilized by adding drops of a diluted sperm. Embryos were cultured at room temperature under gentle agitation with a motor-driven plastic paddle (60 rpm) in filtered seawater. The embryos were observed with a light microscope Biolam (LOMO, S.-Petersburg, Russia). For treatment with the test compounds, 5 mL aliquots of embryo suspension were transferred to 6-well plates and incubated as a monolayer at a concentration up to 2000 embryos/mL. Stock solutions of compounds were prepared in DMSO at 10 mM concentration, followed by a 10-fold dilution with 96% EtOH. This procedure enhanced solubility of the test compounds in the salt-containing medium (seawater), as evidenced by microscopic examination of the samples. The maximal tolerated concentrations of DMSO and EtOH in the *in vivo* assay were determined to be 0.05% and 1%, respectively. Higher concentrations of either DMSO (≥0.1%) or EtOH (>1%) caused non-specific alteration and retardation of the sea urchin embryo development independent of the treatment stage. Combretastatins A-4 and A-2 served as reference compounds.

The antiproliferative activity was assessed by exposing fertilized eggs (8–20 min after fertilization, 43–55 min before the first mitotic cycle completion) to 2-fold decreasing concentrations of the compound. Cleavage alteration and arrest were clearly detected at 2.5–5.5 h after fertilization. The effects were quantitatively estimated as effective threshold concentrations (EC) resulting in cleavage alteration or full mitotic arrest. At these concentrations, all tested microtubule destabilizers caused 100% cleavage alteration and embryo death before hatching, whereas at 2-fold lower concentrations, the compounds failed to produce any effect. For microtubule destabilizing activity, the compounds were tested on free-swimming blastulae just after hatching (9–10 h after fertilization), originated from the same embryo culture. Embryo spinning was observed after 15 min to 20 h of treatment, depending on the structure and concentration of the compound. Both spinning and lack of forward movement were interpreted to be the result of the microtubule destabilizing activity of a molecule. Video illustrations are available at http://www.chemblock.com.

Both sea urchin embryo assay and DTP NCI60 cell line activity data are available free of charge *via* the Internet at http://www.zelinsky.ru.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2013.12.006. These data include MOL files and InChiKeys of the most important compounds described in this article.

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