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A novel application of DDQ as electrophile in the Nenitzescu reaction

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ABSTRACT

Reaction of 2,3-dichloro-5,6-dicyano-benzoquinone (DDQ) with secondary enaminones yields surprisingly 2-aza-spiro[4,5]decatrienes. The reaction occurs via cyclisation of the primary Michael-adduct with the nitrile group. Reaction of DDQ with tertiary and also certain secondary enamines leads to 3-amino-benzo[*b*]furan derivatives. This is formed not by Michael-addition, but via geminate radical ion pair formation with subsequent generation of an oxygen–carbon bond to yield benzofurans. The new products are investigated with regards to inhibition of purified human proteinkinase CK2 and their general cytostatic activity. It turned out, that the most active compound is the 3-amino-5-hydroxy-benzofuran derivative **11s** with an IC_{50} value of 0,2 μM for CK2.

1. Introduction

In continuation of our previous work studying the reaction of quinones and enamines in the Nenitzescu reaction in order to synthesize potential heterocyclic antitumour compounds,[1] we used completely substituted quinones (e.g., **1**) as educt in the reaction with *secondary* enamines **2** leading to the indole **3**[2] (Scheme 1).

Furthermore, we investigated[2] the reaction of the activated quinone **1** and *tertiary* enamines **2**. This yielded indenes **4** by cyclisation via enaminemethyl group due to participation of the methyl group in enamine tautomerization (Scheme 1).

Our idea now was to use DDQ **5a** as a completely substituted and activated quinone in the reaction with enamines. This seemed to be interesting since DDQ, which is normally used as oxidant,[3] is known to react with nucleophilic indoles and enols by C–C-bond formation via Michael-addition.[4,5]

2. Chemical results and discussion

2.1. Secondary enamines

We[6] investigated the reaction of DDQ **5a** and also 2,3-dicyanobenzoquinone **5b** with enamines **2a–n** derived from β-aminocrotonates or β-aminocinnamates, normally used in the Nenitzescu reaction as enamines to yield 5-hydroxy-indoles.[7] The first step should be a Michael-addition to intermediate **6**. After cyclisation in the case of **5a** we expected the formation of dihydroindoles instead of aromatic indoles. Only reaction of quinone **5b** can lead to an aromatic indole.

The products of the reaction with **5a** were isolated in high yield. The spectroscopic investigation showed unexpectedly sp^3-singlets at 89–102 ppm in the ^{13}C NMR-spectra. The shift is in the typical range for a carbinolamine carbon. However, there is only one sp^3-singlet in the ^{13}C NMR-spectra, which is not in agreement with the expected dihydroindole structure.

We were able to acetylate the reaction products (**7**) from the quinones with **2a** and **2h** and obtained the corresponding acetyl derivatives to yield **8a**,**h**. Some crystals from **8a** were suitable for X-ray-analysis. The result (Fig. 1) confirms the correct structure **8a**. Thus, structures **7a–n** are correct for all products, which is shown by the spectroscopic data accordingly.

Thus, the reaction of quinones **5a**,**b** involves in the first step a normal soft–soft reaction of the ß-carbon of enamine **2** in a nucleophilic 1,4-addition to position 2 of the quinone, to yield the intermediate **6** (Scheme 2). The following cyclisation happens by attack of the enamine nitrogen to the neighbouring nitrile group leading to the spirocompounds **7**, being in perfect accordance with the corresponding ^{13}C NMR-data.

To our knowledge, this is the first time, that the formation of a 2-azaspiro[4,5]decatriene as final product of the Nenitzescu reaction has been observed.

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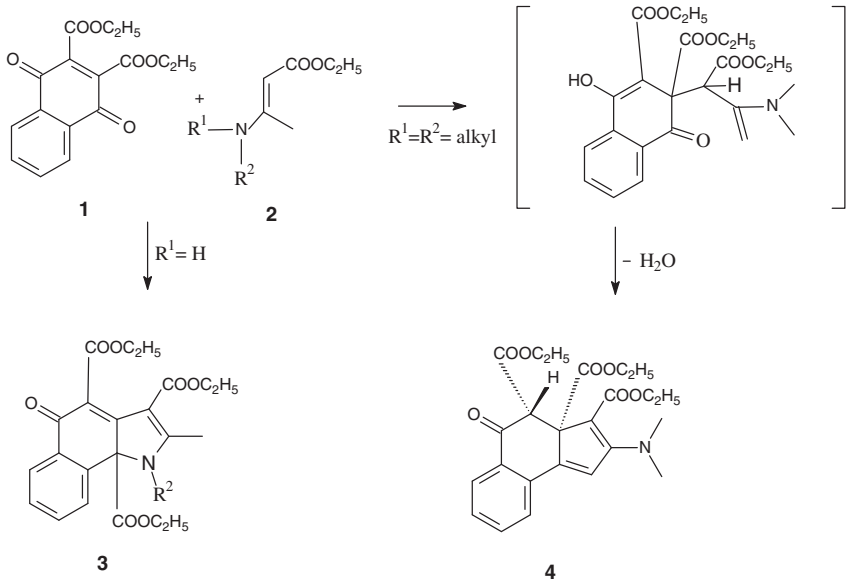


Scheme 1. Reaction of quinone **1** with secondary and tertiary enamines **2**.

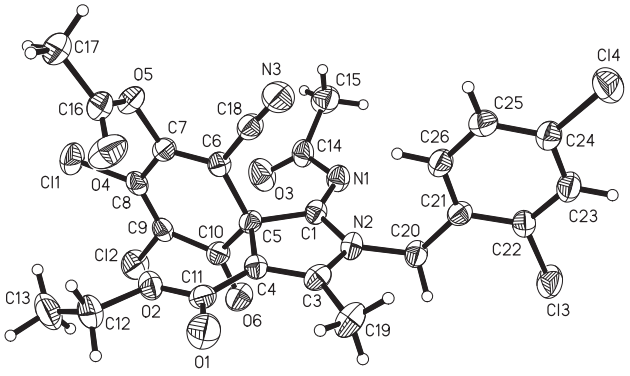


Figure 1. Molecular structure of **8a** in the crystal. Displacement ellipsoids are drawn at the 30% probability level, radii of hydrogen atoms are chosen arbitrarily, and the hydrogen atom labels are omitted for clarity. Compound **8a** is racemic; only one enantiomer is shown. Bond lengths and angles are as to be expected.

2.2. Tertiary enamines

In addition we investigated the reaction of tertiary enamines **2o**,**p** and DDQ **5a**. Based on earlier experience,[2] we expected a benzindene as product according to structure **4** (Scheme 1). The reaction proceeds exothermically with the intermediate appearance of a deep green-blue colour, indicating a possible appearance of a CT-complex.

According to spectroscopic data, we yielded the same product from the reaction of **2o** and **2p** with DDQ. It clearly shows loss of the amine derivative and the acetyl moiety of the parent ß-dicarbonyl compound structure. Thus, no benzindene was synthesized.

The structure **9a** of the product finally was determined by X-ray diffraction (Fig. 2) again. Some chemical transformations were done, too: acetylation to **11a** and hydrogenation to **10** including loss of chlorine and reduction of the nitrile group. Acetylation of **10** yields triacetate **12**.

Enamine **2s** and DDQ led to the corresponding benzofuran **9s**, characterised after acetylation to **11s** (Scheme 3).

Based on the experience of Tanamura[4] and Bhattacharya[5] with DDQ and indoles or enols, it seems reasonable to explain the current unusual path of the reaction to **9** as shown in Scheme 4.

We interpret the reaction sequence as follows (Scheme 4):

1. formation of a CT-complex,
2. SET from enaminone **2** to DDQ **5**,
3. formation of a radicalion pair,
4. radical combination,
5. ring closure of the CH-acidic intermediate with the nitrile group,
6. hydrolytic cleavage to 3-amino-benzofuran with loss of dialkylacetamid and formation of the final product **9**.

This interpretation of the reaction is supported by the observation, that benzofuran **14** can be isolated after reaction of DDQ with the cyclic enaminone **13** (Scheme 5).

In this case the cyclohexanone ring is opened and due to the cyclic structure the amid moiety remains bounded to the benzofuran structure in **14**.

The formation of 5-hxdroxybenzofurans in the Nenitzescu reaction, especially with tertiary enaminones is known since long time,[8] however, this happens without cleavage of the enaminone structure.

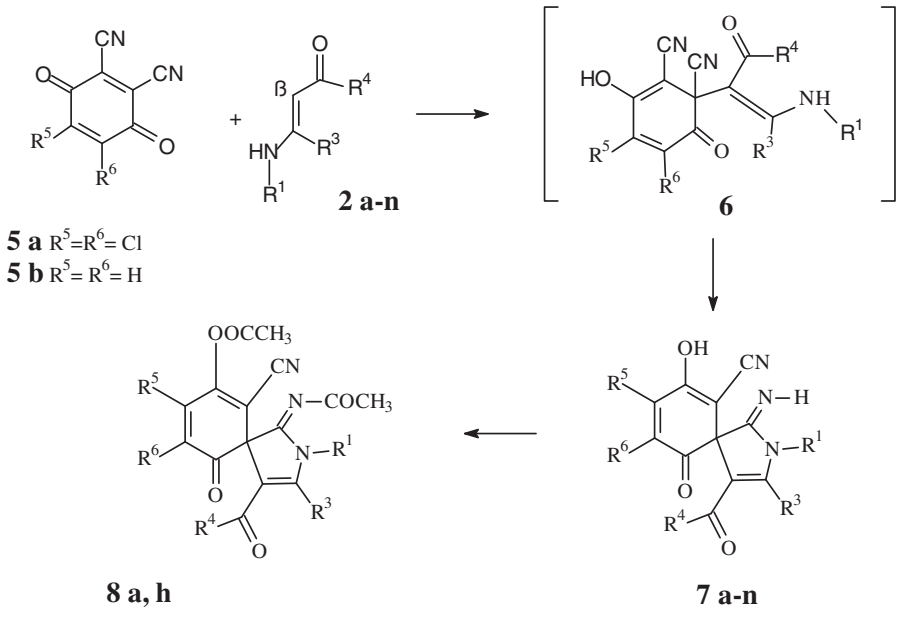
In order to get further insight into the structure–activity relationship (SAR) of biological activity of the new benzofurans, we synthesized (Scheme 6) a similar benzofuran in this manner with no amino group in position 2 starting from benzoquinone and enamine **2s** to yield **15**.

3. Biological results

Spirocompounds **7a**,**c**,**f** and **j** were tested against human tumour cell lines in vitro at the NCI.[9,10] The mean graph mid point (MGM): $\log_{10} GI_{50}$ (averaged log mol concentration for all tested 60 cell lines which led to 50% growth inhibition) is in the region from −4.1 to −4.3; correspondingly they show weak cytostatic activity.

However, for some subpanel cell lines high activity (ovary, mamma, prostate and lung) is observed: MGM $\log_{10} GI_{50}$ for **7a** (OVCR5: −5.57, NCI/ADRRES: −6.72), **7c** (DU-145: −6.47), **7f** (HOP-92: −5.75), **7j** (HOP-92: −6.26).

Since it is known,[11,12] that the expression and activity of proteinkinase CK2 in many tumour cells are increased and thus apoptose of these cells is inhibited, proteinkinase CK2 inhibitors



7	R^1	R^3	R^4	R^5=R^6
a	2,4-dichlorobenzyl	Me	OC_2H_5	Cl
b	4-tolyl	Me	OC_2H_5	Cl
c	4-chlorophenyl	Me	OC_2H_5	Cl
d	4-bromophenyl	Me	OC_2H_5	Cl
e	2-isobutyl	Me	OC_2H_5	Cl
f	2,4-difluorophenyl	Me	OC_2H_5	Cl
g	4-methoxyphenyl	Me	OC_2H_5	Cl
h	benzyl	phenyl	OC_2H_5	Cl
i	4-fluorbenzyl	phenyl	OC_2H_5	Cl
j	4-methoxyphenyl	phenyl	OC_2H_5	Cl
k	phenyl	Me	phenyl	Cl
l	4-tolyl	Me	OC_2H_5	H
m	4-bromphenyl	Me	OC_2H_5	H
n	4-methoxyphenyl	Me	OC_2H_5	H
8				
a	2,4-dichlorobenzyl	Me	OC_2H_5	Cl
h	benzyl	phenyl	OC_2H_5	Cl

Scheme 2. Reaction of quinones **5a** and **5b** with secondary enamines to spirocompounds **7** and **8**.

can be useful antitumour agents. Thus we exemplarily investigated CK2 inhibition of spirocompound **8b** by a previously established CE assay.[13] However, there was no inhibition at a concentration of 10 μM. In contrast, significant inhibition of human proteinkinase CK2 at low concentrations was found for the benzofuran **9s** and its acetyl derivative **11s** (Table 2). It seems, that a keto group in position 2 and a free amino group in position 3 of benzo[*b*]furan are necessary for the CK2 inhibitory activity. As is shown in Figure 2 and Table 1 for **9a**, the new benzofurans are able to interact with molecules via hydrogen bonds; may be, this is responsible for inhibition of proteinkinase CK2.

4. Conclusion

DDQ is able to react not only as oxidant but as electrophile with the ring carbon (1,4-addition) as well as with nitrile carbon. The reaction path with DDQ and enaminones is quite different from similar diethyl naphthoquinone-2,3-dicarboxylate due to higher reactivity of the nitrile group and formation of a semiquinone radical anion. New, before unexpectable, aminobenzofurans and heterocyclic spiro-compounds are accessible now. Another new variation of the initial Nenitzescu reaction and new aspects in radical chemistry of push–pull-enamines are found.

Moreover, new lead structures are discovered in the field of anticancer drug development. Up to now spirocompounds **7** seem to be useful as lead structures to develop new anticancer agents by further variation of the structure and the substituents. In the benzofuran series a very potent in vitro antitumour structure with high inhibition of human proteinkinase CK2 is discovered.

5. Experimental

5.1. General methods

Melting points were taken on Gallenkamp apparatus and are uncorrected. IR spectra were recorded as frequency in cm^{-1} on a Perkin–Elmer 1600 series FT-IR spectrometer in KBr. ^1H and ^{13}C NMR-spectra were recorded using Bruker AC-200 instrument (200 and 50 MHz), shift in ppm, coupling constants in Hz. UV/vis spectra were recorded on Perkin–Elmer Lamda 16 λ_{max} nm (log ε). Mass spectra were performed with Finnigan MAT 8200 or MAT 311A (EI, 70 eV) *m*/*z* (% rel. intensity). Microanalysis was performed on Perkin–Elmer PE2400 CHN analysator.

Crystals of **8a** and **9a** × DMSO suitable for X-ray study were selected by means of a polarisation microscope and investigated on a STOE Imaging Plate Diffraction System using graphite monochromatized MoKα radiation (λ = 0.71073 Å).[14] To avoid loss of DMSO and deterioration, the crystal of **13a** × DMSO had to be enclosed in a thin walled glass capillary Unit cell parameters were determined by least-squares refinements on the positions of 8000

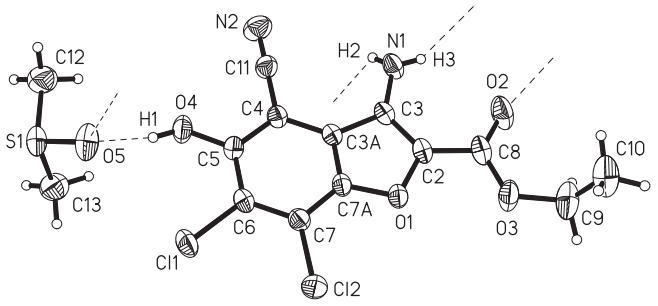


Figure 2. Diagram of the asymmetric unit of the crystal structure of **9a** × DMSO. Displacement ellipsoids are drawn at the 30% probability level, radii of hydrogen atoms are chosen arbitrarily, and the hydrogen atom labels are omitted for clarity. Bond lengths and angles are as to be expected. Dashed lines are indicating intra- and intermolecular hydrogen bonds.

reflections in the range $1.45° < \theta < 25.85°$ and $2.00° < \theta < 26.00°$, respectively. Space group type No. 14 was uniquely determined in both cases. Corrections for Lorentz and polarisation effects were applied. The structures were solved by direct methods[15] and subsequent ΔF-syntheses. Approximate positions of all the hydrogen atoms were found in different stages of refinements by full-matrix least-squares calculations on F^2. [16] Anisotropic displacement parameters were refined for all non-hydrogen atoms. For the H atoms at N1, and O3 of **9a** × DMSO positional and isotropic displacement parameters were refined. With idealised bonds lengths and angles assumed for all the CH_3, CH_2, and CH groups of both compounds, the riding model was applied for the corresponding H atoms. In addition, the H atoms of the CH_3 groups were allowed to move collectively around the neighbouring C–C axis. The isotropic displacement parameters of the H atoms were constrained to 120% of the equivalent isotropic displacement parameters of the parent C atoms for the CH and CH_2 groups and equal to 150% for the CH_3 groups.

5.2. General procedure for the preparation of the ethyl-6-cyano-7-hydroxy-1-imino-10-oxo-2-aza-spiro[4.5]deca-3,6,8-triene-4-carboxylates 7a–n

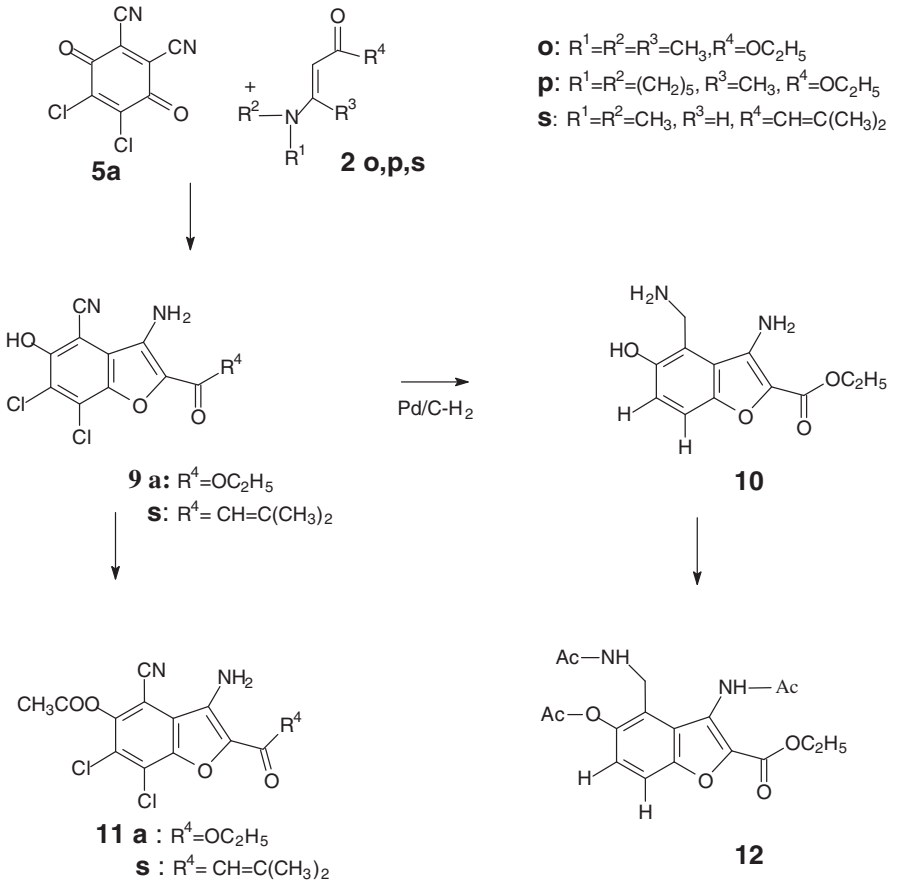
To a stirred solution of the appropriate enaminone **2a**–**n** in glacial acetic acid, acetone or nitromethane was added 2,3-dichlor-5,6-dicyano-*p*-benzoquinone (DDQ) **5a** or 2,3-dicyano-*p*-benzoquinone **5b**. The mixture was stirred for 2–4 h. The precipitation was isolated and washed with diethylether or acetone.

5.2.1. Ethyl-8,9-dichloro-6-cyano-7-hydroxy-1-imino-2-(2,4-dichlorobenzyl)-3-methyl-10-oxo-2-aza-spiro[4.5]deca-3,6,8-triene-4-carboxylate 7a

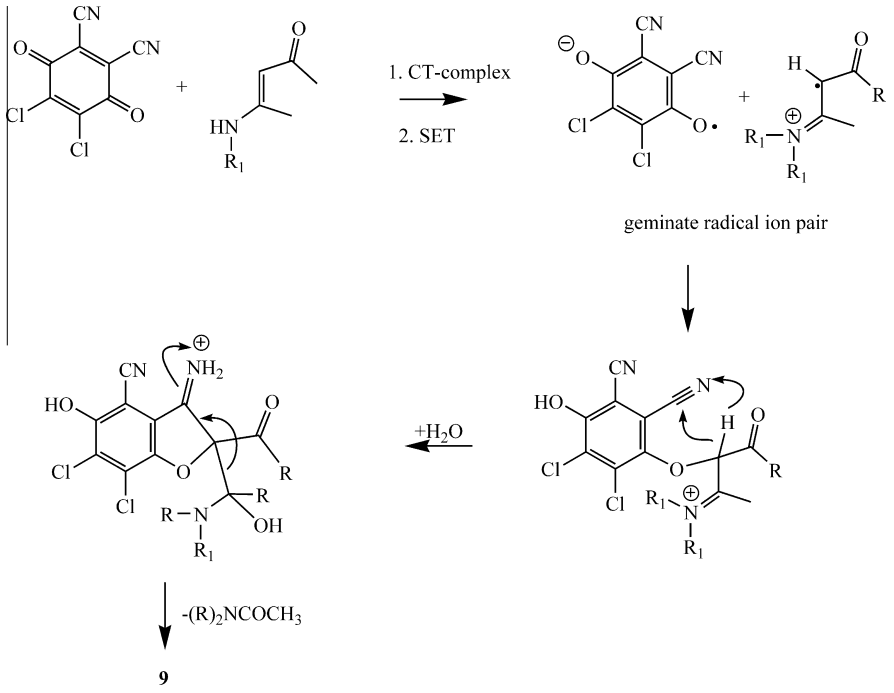
474 mg DDQ **5a** (2.09 mmol), 500 mg (1.74 mmol) **2a**, 5 ml glacial acetic acid, 646 mg (62%), mp 171 °C (orange powder from glacial acetic acid). IR 2186, 1690. ^{1}H NMR (DMSO-d_6) δ 11.27–10.23(br s, 2H, =NH, OH), 7.52–7.31 (m, 4H, arom. H), 5.21 (s, 2H, CH_2), 4.06 (q, J = 7.0 Hz, 2H, $COOCH_2CH_3$), 2.16 (s, 3H, CH_3), 1.08 (t, J = 7.0 Hz, 3H, $COOCH_2CH_3$). MS (FAB+NBA) 515 (22, M^+), 468 (11), 442 (5), 413 (7), 354 (9), 329 (43), 307 (100). Anal. Calcd for $C_{21}H_{15}Cl_4N_3O_4$ (515.17): C, 48.16; H, 2.93; N, 8.16. Found: C, 48.11; H, 2.78; N, 7.96.

5.2.2. Ethyl-8,9-dichloro-6-cyano-7-hydroxy-1-imino-2-(4-tolyl)-3-methyl-10-oxo-2-aza-spiro[4.5]deca-3,6,8-triene-4-carboxylate 7b

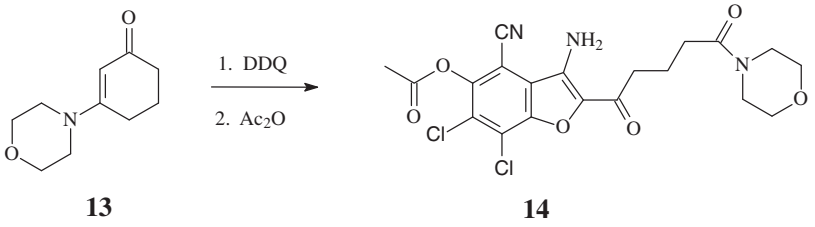
995 mg DDQ **5a** (4.38 mmol), 800 mg (3.65 mmol) **2b**, 8 ml glacial acetic acid, 1.25 g (77%), mp 302 °C (orange powder from



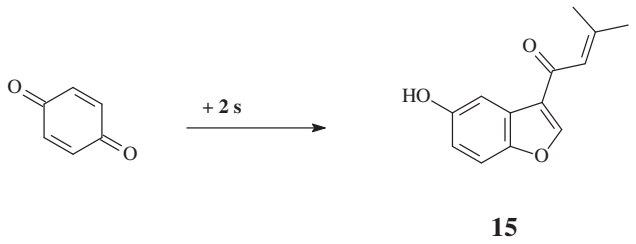
Scheme 3. Reaction of DDQ (**5a**) and tertiary enamines to 3-amino-benzofurans **9**.



Scheme 4. Path of the reaction to 3-amino-benzofurans **9**.



Scheme 5. Reaction of the cyclic enamine **13** with DDQ **5a**.



Scheme 6. Synthesis of benzofuran **15** from *p*-benzoquinone and enamine **2s**.

Table 1
Hydrogen bonds for **9a** (Å and °)

D-H···A	d(D-H)	d(H···A)	d(D···A)	<(DHA)
O(4)-H(1)···O(5)	0.79(5)	1.80(6)	2.580(4)	169(5)
N(1)-H(2)···O(5)#1	0.81(2)	2.43(3)	3.003(3)	128(3)
N(1)-H(3)···O(2)	0.83(2)	2.38(3)	2.932(4)	124(3)
N(1)-H(3)···O(2)#2	0.83(2)	2.25(4)	2.954(4)	143(3)

Symmetry transformations used to generate equivalent atoms:
#1 $x-1, y, z$.; #2 $-x-1, -y+1, -z+2$.

Table 2
CK2-inhibition of products **8–15**

	CK2-Inhibition (10 mM)	IC_{50} (μM)
9s	>96%	0.2
11s	93%	0.2
11a	69%	2.4
15	39%	n.d.
12	0%	n.d.
8a	0%	n.d.

It is worth mentioning, that the CK2 enzyme inhibitor **11s** at a concentration of 27 μM shows a growth inhibition of more than 99% for human lymphoma cell lines as well (private communication from Prof. Dr. W.E.G. Mueller, Institute for Physiological Chemistry and Pathobiochemistry, Universität Mainz, D55099 Mainz, Duesbergweg 6).

glacial acetic acid). IR 2182, 1703, 1674. ^{1}H NMR (DMSO-d_6) δ 11.24 (br s, 1H, =NH), 10.18 (br s, 1H, OH), 7.52–7.31 (m, 4H, arom. H), 4.06 (q, J = 7.0 Hz, 2H, $COOCH_2CH_3$), 2.42 (s, 3H, 4′–CH_3), 2.16 (s, 3H, CH_3), 1.08 (t, J = 7.0 Hz, 3H, $COOCH_2CH_3$). MS 445 (16, M^+), 399 (55), 374 (63), 319 (30), 267 (93), 132 (60), 91 (100). Anal. Calcd for $C_{21}H_{17}Cl_2N_3O_4$ (446.29): C, 56.52; H, 3.84; N,9.42. Found: C,56.38; H, 3.80; N, 9.20.

5.2.3. Ethyl-8,9-dichloro-6-cyano-7-hydroxy-1-imino-2-(4-chlorophenyl)-3-methyl-10-oxo-2-azaspiro [4.5]deca-3,6,8-triene-4-carboxylate 7c

912 mg DDQ **5a** (4.01 mmol), 800 mg (3.34 mmol) **2c**, 5 ml glacial acetic acid, 1.32 g (85%), mp 227 °C (yellow-orange powder from glacial acetic acid). IR 2184, 1699, 1677. ^{1}H NMR (DMSO-d_6) δ 11.36 (br s, 1H, =NH), 10.42 (br s, 1H, OH), 7.98–7.16 (m, 4H, arom. H), 4.13 (q, J = 7.0 Hz, 2H, $COOCH_2CH_3$), 2.25 (s, 3H,

CH_3), 1.15 (t, J = 7.0 Hz, 3H, $COOCH_2CH_3$), MS 465 (40, M^+), 436 (47), 419 (61), 395 (53), 329 (42), 313 (26), 267 (100), Anal. Calcd for $C_{20}H_{14}Cl_3N_3O_4$ (466.71): C, 51.47; H, 3.02; N, 9.00. Found: C, 51.00; H, 2.94; N, 8.85.

5.2.4. Ethyl-8,9-dichloro-6-cyano-7-hydroxy-1-imino-2-(4-bromophenyl)-3-methyl-10-oxo-2-aza-spiro[4.5]deca-3,6,8-triene-4-carboxylate 7d

1.1 g DDQ **5a** (4.84 mmol), 1.1 g (4.04 mmol) **2d**, 5 ml glacial acetic acid, 1.5 g (71%), mp 230 °C (yellow-orange powder from glacial acetic acid). IR 2181, 1697, 1673. ^{1}H NMR (DMSO-d_6) δ 11.33 (br s, 1H, =NH), 10.39 (br s, 1H, OH), 8.05–7.06 (m, 4H, arom. H), 4.08 (q, J = 7.0 Hz, 2H, $COOCH_2CH_3$), 2.27 (s, 3H, CH_3), 1.10 (t, J = 7.0 Hz, 3H, $COOCH_2CH_3$). MS 511 (45, M^+), 482 (10), 430 (7), 314 (24), 267 (32), 228 (68), 198 (35), 87 (16), 40 (22), 35 (100). Anal. Calcd for $C_{20}H_{14}BrCl_2N_3O_4$ (511.16): C, 47.00; H, 2.76; N, 8.22. Found: C, 46.86; H, 2.41; N, 8.07.

5.2.5. Ethyl-8,9-dichloro-6-cyano-7-hydroxy-1-imino-2-isobutyl-3-methyl-10-oxo-2-aza-spiro[4.5]deca-3,6,8-triene-4-carboxylate 7e

1.9 g DDQ **5a** (8.37 mmol), 1.3 g (7.01 mmol) **2e**, 8.5 ml glacial acetic acid, 2.65 g (92%), mp 174 °C (orange powder from glacial acetic acid). IR 2190, 1698, 1682. ^{1}H NMR (DMSO-d_6) δ 10.93 (br s, 1H, =NH), 10.54 (br s, 1H, OH), 4.03 (q, J = 7.0 Hz, 2H, $COOCH_2CH_3$), 3.67 (d, J = 7.5 Hz, CH_2), 2.53 (s, 3H, CH_3), 2.02 (m, 1H, CH), 1.07 (t, J = 7.0 Hz, 3H, $COOCH_2CH_3$), 0.94 (m, 6H, 2 × CH_3–isobutyl), MS 411 (26, M^+), 327 (41), 282 (39), 267 (100). Anal. Calcd for $C_{18}H_{19}Cl_2N_3O_4$ (412.28): C, 52.44; H, 4.65; N, 10.19 Found: C, 52.34; H, 4.86; N, 9.80.

5.2.6. Ethyl-8,9-dichloro-6-cyano-7-hydroxy-1-imino-2-(2,4-difluorophenyl)-3-methyl-10-oxo-2-aza-spiro[4.5]deca-3,6,8-triene-4-carboxylate 7f

1.921 g DDQ **5a** (8.46 mmol), 1.7 g (7.05 mmol) **2f**, 5 ml glacial acetic acid, 2.43 g (74%), mp 212 °C (yellow-orange powder from glacial acetic acid). IR 2186, 1702, 1672. ^{1}H NMR (DMSO-d_6) δ 11.54–8.32 (br s, 2H, =NH, –OH), 7.87–7.22 (m, 3H, arom H), 4.08 (q, J = 7.0 Hz, 2H, $COOCH_2CH_3$), 2.20 (s, 3H, CH_3), 1.10 (t, J = 7.0 Hz, 3H, $COOCH_2CH_3$). MS 467 (32, M^+), 440 (24), 386 (12), 317 (5), 228 (65), 154 (100), Anal. Calcd for $C_{20}H_{13}Cl_2F_2N_3O_4$ (468.25): C, 51.30; H, 2.80; N, 8.97. Found: C, 51.12; H, 2.62; N, 9.17.

5.2.7. Ethyl-8,9-dichloro-6-cyano-7-hydroxy-1-imino-2-(4-methoxyphenyl)-3-methyl-10-oxo-2-aza-spiro[4.5]deca-3,6,8-triene-4-carboxylate 7g

930 mg DDQ **5a** (3.93 mmol), 770 mg (3.27 mmol) **2g**, 5 ml glacial acetic acid, 1.13 g (75%), mp 238 °C, (orange powder from glacial acetic acid). IR 2179, 1703, 1673. ^{1}H NMR (DMSO-d_6) δ 11.42 (s, br s, 1H, =NH),10.19 (s, br s, 1H, OH), 7.57–7.05 (m, 4H, arom. H), 4.07 (q, J = 7.0 Hz, 2H, $COOCH_2CH_3$), 3.68 (s, 3H, OCH_3), 2.18 (s, 3H, CH_3), 1.09 (t, J = 7.0 Hz, 3H, CH_3). MS 461 (15, M^+), 416 (25), 398 (10), 388 (20), 335 (34), 317 (13), 267 (46), 148 (100). Anal. Calcd for $C_{21}H_{17}Cl_2N_3O_5$ (462.29): C, 54.56; H, 3.71; N, 9.09. Found: C, 54.36; H, 3.58; N, 9.00

5.2.8. Ethyl-8,9-dichloro-6-cyano-7-hydroxy-1-imino-2-benzyl-10-oxo-3-phenyl-2-aza-spiro[4.5]deca-3,6,8-triene-4-carboxylate 7h

234 mg DDQ **5a** (1.03 mmol), 300 mg (0.86 mmol) **2h**, 3 ml glacial acetic acid, 310 mg (71%), mp 173 °C (orange powder from glacial acetic acid). IR 2190, 1706, 1686. ^{1}H NMR (DMSO-d_6) δ 11.24 (s, br s, 1H, =NH), 10.85 (s, br s,1H, OH), 7.58–6.97 (m, 10H, arom H), 4.89 (q, 2H, CH_2–benzyl), 4.01–3.72 (q, J = 7.0 Hz, 2H, $COOCH_2CH_3$), 0.81 (t, J = 7.0 Hz, 3H, $COOCH_2CH_3$). MS (FAB + NBA) 508 (M^++1). $C_{26}H_{19}Cl_2N_3O_4$ (508.36).

5.2.9. Ethyl-8,9-dichlor-6-cyano-7-hydroxy-1-imino-2-(4-fluorbenzyl)-10-oxo-3-phenyl-2-aza-spiro[4.5]deca-3,6,8-trien-4-carboxylate 7i

1.36 g (6.01 mmol) DDQ **5a**, 1,5 g (5,01 mmol) **2i**, 6 ml glacial acetic acid, 1,13 g (43%), mp168 °C (orange powder). IR 2184, 1684. ^{1}H NMR (DMSO-d_6) δ 11.26 (br s, 1H, =NH), 10.91 (br s, 1H, OH), 7.68–6.94 (m, 9H, arom. H), 4.87 (s, 2H, CH_2), 3.86 (q, J = 7.0 Hz, 2H, $COOCH_2$), 0.82 (t, J = 7.0 Hz, 3H, $COOCH_2CH_3$). MS 525 (2; M^+), 331 (8), 375 (5), 347 (4), 330 (6), 329 (7), 109 (100). Anal. Calcd for $C_{26}H_{18}Cl_2FN_3O_4$ (526.35): C, 59.37; H, 3.45; N, 7.98. Found.: C, 59.41; H, 3,22; N, 7,66.

5.2.10. Ethyl-8,9-dichloro-6-cyano-7-hydroxy-1-imino-2-(4-methoxyphenyl)-10-oxo-3-phenyl-2-aza-spiro[4.5]deca-3,6,8-triene-4-carboxylate 7j

274 mg DDQ **5a** (1.21 mmol), 300 mg (1.00 mmol) **2j**, 3 ml glacial acetic acid, 266 mg (51%), mp 220 °C (orange-red powder from glacial acetic acid). IR 2185, 1704, 1670. ^{1}H NMR (DMSO-d_6) δ 11.38 (br s, 1H, =NH), 10.23 (br s,1H, OH), 7.53–6.78 (m, 9H, arom H), 4.89 (s, 3H, OCH_3), 3.89 (q, J = 7.0 Hz, 2H, $COOCH_2CH_3$), 0.85 (t, J = 7.0 Hz, 3H, $COOCH_2CH_3$). MS 523 (49, M^+), 495 (87), 374 (92), 329 (100). Anal. Calcd for $C_{26}H_{19}Cl_2N_3O_5$ (524.36): C,59.56; H, 3.56; N, 8.01. Found: C, 59.33; H, 3.44; N, 7.90.

5.2.11. 4-Benzoyl-8,9-dichlor-6-cyano-7-hydroxy-1-imino-2-phenyl-10-oxo-3-phenyl-2-aza-spiro[4.5]deca-3,6,8-trien 7k

575 mg (2,53 mmol) DDQ **5a**, 500 mg (2,1 mmol) **2k**, 2 ml glacial acetic acid, 185 mg (19%), mp 130 °C, orange powder. IR 2179, 1682. ^{1}H NMR (DMSO-d_6) δ 11.23 (br s, 1H, NH), 10.22 (s, 1H, OH), 7.84–7.33 (m, 10H, arom. H), 1.65 (s, 3H, CH_3). MS 464 (6; M^+), 393 (87), 345 (38), 268 (13), 183 (9), 118 (60), 77 (100), 51 (58). Anal. Calcd for $C_{24}H_{15}Cl_2N_3O_3$ (464,30): C: 62,08; H: 3,26; N: 9,05. found: C, 61.83; H, 3.28; N, 8,65.

5.2.12. Ethyl-6-cyano-7-hydroxy-1-imino-2-(4-tolyl)-3-methyl-10-oxo-2-aza-spiro[4.5]deca-3,6,8-triene-4-carboxylate 7l

300 mg 2,3-dicyan-*p*-benzoquinone **5b** (1.89 mmol), 202 mg (1.89 mmol) **2l**, 3 ml nitromethan, 434 mg (61%), mp 197 °C (yellow powder from nitromethan). IR 2185, 1704, 1670. ^{1}H NMR (DMSO-d_6) δ 11.65 (s, br s, 1H, =NH), 8.56 (s, br, 1H, OH), 7.44–6.98 (m, 4H, arom H's), 7.23 (d, J = 10.1 Hz, 1H, H-8), 6.41 (d, J = 10.1 Hz, 1H, H-7), 4.1 (mc, 2H, $COOCH_2CH_3$), 2.37 (s, 3H, CH_3), 2.18 (s, 3H, CH_3), 1.09 (t, J = 7.0 Hz, 3H, $COOCH_2CH_3$). MS 377 (100, M^+), 349 (21), 331 (40), 320 (15), 276 (40), 223 (32), 132 (67). Anal. Calcd for $C_{21}H_{19}N_3O_4$ (377.40): C, 66.83; H, 5.07; N,11.13. Found: C, 66.63; H, 4.80; N, 10.86.

5.2.13. Ethyl-6-cyano-7-hydroxy-1-imino-2-(4-bromophenyl)-3-methyl-10-oxo-2-aza-spiro[4.5]deca-3,6,8-triene-4-carboxylate 7m

300 mg 2,3-dicyan-*p*-benzoquinone **5b** (1.89 mmol), 325 mg (1.89 mmol) **2m**, 1.5 ml nitromethan and 0.75 ml glacial acetic acid, 343 mg (42%), mp180 °C (yellow powder from nitromethan). IR 2193, 1693, 1666. ^{1}H NMR (DMSO-d_6) δ 11.74 (s, br s, 1H, =NH), 8.74 (s, br, 1H, OH), 7.84–7.09 (m, 4H, arom H), 7.24 (d, ^2J = 10.2 Hz, 1H, H-8), 6.41 (d, ^2J = 10.2 Hz, 1H, H-7), 4.1 (mc, 2H, $COOCH_2CH_3$), 2.21 (s, 3H, CH_3), 1.09 (t, J = 7.0 Hz, 3H, $COOCH_2CH_3$). MS 443 (35, M^+), 397 (85), 355 (46), 300 (36), 245 (33), 199 (100), Anal. Calcd for $C_{20}H_{16}BrN_3O_4$ (442.27): C, 54.32; H, 3.65; N, 9.50. Found: C, 53.90; H, 3.36; N, 9.26.

5.2.14. Ethyl-6-cyano-7-hydroxy-1-imino-2-(4-methoxyphenyl)-3-methyl-10-oxo-2-aza-spiro[4.5]deca-3,6,8-triene-4-carboxylate 7n

300 mg 2,3-dicyan-*p*-benzoquinone **5b** (1.89 mmol), 446 mg (1.89 mmol) **2n**, 5 ml glacial acetic acid, 355 mg (48%), mp102 °C (yellow powder from glacial acetic acid). IR 2188, 1693, 1682. ^{1}H NMR (DMSO-d_6) δ 11.69 (s, br s, 1H, =NH), 8.64 (s, br s, 1H, OH), 7.23 (d, ^2J = 10.1 Hz, 1H, H-8), 6.42 (d, ^2J = 10.1 Hz, 1H, H-7) 7.18–7.02 (m, 4H, arom H's), 4.1 (mc, 2H, $COOCH_2CH_3$), 3.81 (s, 3H, OCH_3), 2.18 (s, 3H, CH_3), 1.10 (t, J = 7.0 Hz, 3H, $COOCH_2CH_3$). ^{13}C NMR (50 MHz, DMSO-d_6) δ 193.1 (s, C-10), 165.1 (s, C-7), 162.2 (s, C=O, ester), 159.7 (s, C-4′), 159.4 (s, C-1), 159.1 (s, C-3), 140.2 (d, J = 163.9 Hz, C-8), 130.4 (d, J = 167.4 Hz, C-2′, C-6′), 129.8 (d, J = 167.8 Hz, C-3′, C-5′), 126.1 (s, C-9), 115.6 (s, –CN), 115.1 (d, J = 162.3 Hz, C-9), 107.3 (s, C-4), 89.0 (s, C-6), 61.4 (s, C-5), 59.3 (t, $COOCH_2CH_3$), 55.3 (q, OCH_3–), 13.9 (q, CH_3), 13.2 (q, $COOCH_2CH_3$). MS 393 (66, M$^+$), 351 (43), 292 (55), 264 (21), 199 (51), 148 (100). Anal. Calcd for $C_{21}H_{19}N_3O_5$ (393.40): C, 64.12; H, 4.87; N, 10.68. Found: C, 63.83; H, 4.68; N, 10.45.

5.3. General procedure for the preparation of the ethyl-8,9-dichloro-6-cyano-7-acetoxy-1-acetylimino-10-oxo-2-aza-spiro[4.5]deca-3,6,8-triene-4-carboxylates

To a stirred suspension of the appropriate compound **7** was added 1.5 ml acetic anhydride and 0.05 ml diazabicycloundecan (DBU). After stirring for one hour by 70 °C the solvent was removed in vacuo and the residue was recrystallized from diethylether/petrolether.

5.3.1. Ethyl-8,9-dichloro-6-cyano-7-acetoxy-1-acetylimino-2-(2,4-dichlorobenzyl)-3-methyl-10-oxo-2-aza-spiro[4.5]deca-3,6,8-triene-4-carboxylate 8a

500 mg (0.97 mmol) **7a**, 1.5 ml acetic anhydrid, 0.05 ml DBU, 505.70 mg (87%), mp 142 °C (yellow crystals from chloroform/petrolether), IR 2221, 1798, 1697, 1675, 1639, ^{1}H NMR (DMSO-d_6) δ 7.55 (s, 1H, H-3′), 7.40–7.35 (m, 2H, H-4′, H-5′), 5.37 (d, J = 17.1 Hz, 1H, CH_2), 4.2 (mc, 2H, $COOCH_2CH_3$), 2.51 (s, 3H, $OCOCH_3$), 2.46 (s, 3H, =$NCOCH_3$), 1.24 (t, J = 7.0 Hz, 3H, $COOCH_2CH_3$).

^{13}C NMR (50 MHz, $CDCl_3$) δ 182.4 (s, C-10), 179.5 (s, =$NCOCH_3$), 166.0 (s, $OCOCH_3$), 161 (s, $COOC_2H_5$) 159.9 (s, C-1), 158.6 (s, C-7), 152.9 (s, C-3), 138.5 (s, C-2′), 137.8 (s, C-4′), 134.4 (s, C-9), 132.7 (s, C-1′), 130.6 (d, C-3′), 129.5 (d, C-5′), 128.2 (d, C-6′), 127.8 (s, C-8), 111.8 (s, –CN), 110.1 (s, C-4), 102.0 (s, C-6), 63.2 (C-5), 61.0 (t, $COOCH_2CH_3$), 43.4 (t, N–CH_2), 26.5 (q, =$NCOCH_3$), 20.1 (q, $OCOCH_3$), 13.7 (q, CH_3), 12.34 (q, $COOCH_2CH_3$), MS 599 (29, M$^+$), 555 (29), 513 (18), 396 (12) 354 (11), 313 (6), 159(100). Anal. Calcd for $C_{25}H_{19}Cl_4N_3O_6$ (599.25): C, 50.11; H, 3.20; N, 7.01. Found: C, 50.26; H, 3.12; N, 6.87.

5.3.2. Crystal structure determination of compound 8a

Summary of crystal data, details of intensity measurement and structure refinement of **8**[a]

Empirical formula	$C_{25}H_{19}Cl_4N_3O_6$	
Formula weight	599.23	
Temperature	291(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	$P2_1/c$	
Unit cell dimensions	a = 10.9273(6) Å	α = 90°
	b = 11.1960(6) Å	β = 103.648(7)°
	c = 22.5141(15) Å	γ = 90°
Volume	2676.6(3) Å^3	
Z	4	
Density (calculated)	1.487 mg/m^3	
Absorption coefficient	0.488 mm^{-1}	
F(000)	1224	
Crystal size	0.3 × 0.2 × 0.2 mm^3	
θ-Range for data collection	1.92–25.00°	
Index ranges	$-12 \leqslant h \leqslant 12$, $-13 \leqslant k \leqslant 13$, $-26 \leqslant l \leqslant 26$	
Reflections collected	34147	
Independent reflections	4709 [R(int) = 0.0687]	
Completeness to θ = 25.00°	100.0%	
Absorption correction	None	
Refinement method	Full-matrix least-squares on F^2	
Data/restraints/parameters	4709/0/347	
Goodness-of-fit on F^2	1.048	
Final R indices [I >2σ(I)]	R_1 = 0.0431, wR_2 = 0.0693	
R indices (all data)	R_1 = 0.0838, wR_2 = 0.0720	
Largest diff. peak and hole	0.228 and −0.193 e.Å^{-3}	

5.3.3. Ethyl-8,9-dichloro-6-cyano-7-acetoxy-1-acetylimino-2-benzyl-10-oxo-3-phenyl-2-aza-spiro[4.5]deca-3,6,8-triene-4-carboxylate (*Z*:*E*-isomer: in the ratio of 1:1) 8h

700 mg (1.37 mmol) **7h**, 4 ml acetic anhydride, 178 mg (22%), mp 157 °C (yellow crystals from diethylether). IR 2186, 1690. ^{1}H NMR (DMSO-d_6) δ 7.60–6.93 (m, 10H, arom H), 4.99–4.51 (q, 2H, CH_2–benzyl), 3.8 (mc, 2H, $COOCH_2CH_3$), 2,48 (s, 1.5H, =$NCOCH_3$), 2.17 (s, 1.5H, =$NCOCH_3$), 2.13 (s, 3H, –$OCOCH_3$), 1.90 (s, 3H, –$OCOCH_3$), 0.8 (m, 3H, $COOCH_2CH_3$). MS 591 (2, M$^+$), 549 (7), 507 (10), 416 (15), 375 (14), 91 (100). Anal. Calcd for $C_{30}H_{23}Cl_2N_3O_6$ (592.44): C, 60.82; H, 3.91; N, 7.09. Found: C, 60.93; H, 3.84; N, 7.06.

5.3.4. Ethyl-3-amino-6,7-dichloro-4-cyano-5-hydroxy-benzo[*b*]furan-2-carboxylate 9a

To a stirred solution of the tertiary enaminone in acetic acid was added DDQ in a twenty percent surplus. The mixture was stirred for 2 h. The precipitation was isolated and washed with diethylether. 1830 mg DDQ **5a** (8.08 mmol), 2000 mg Enamin **2o** (6.73 mmol) or **2p**, 12 ml acetic acid, 422 mg (91%), mp 227 °C (white-yellow powder from acetic acid). IR 2227, 1681. ^{1}H NMR (DMSO-d_6) δ 11.66 (s, br s, 1H, OH), 5.59 (s, br s, 2H, –NH_2), 4.33 (q, J = 7.0 Hz, 2H, $COOCH_2CH_3$), 1.32 (t, J = 7.0 Hz, 3H, $COOCH_2CH_3$). ^{13}C NMR (50 MHz, DMSO-d_6) δ 159.6 (s, C-5), 153.8 (s, $COOCH_2CH_3$), 143 (s, C-7a), 137.0 (s, C-3a), 127.0 (s, C-7), 123.1 (s, C-6), 121.9 (s, –CN), 119.4 (s, C-2), 113.8 (s, C-3), 90.1 (s, C-4), 60.2 (t, OCH_2)14.2 (q, OCH_2CH_3). MS 314 (63, M$^+$), 268 (100), 292 (55), 212 (45). Anal. Calcd for $C_{12}H_8Cl_2N_2O_4$: (315.11): C, 45.74; H, 2.56; N, 8.89. Found: C, 45.71; H, 2.60; N, 8.68.

5.3.5. Crystal structure determination of compound 9a

Summary of crystal data, details of intensity measurement and structure refinement of compound **9a** × DMSO

Empirical formula	$C_{14}H_{14}Cl_2N_2O_5S$	
Formula weight	393.23	
Temperature	291(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	$P2_1/n$	
Unit cell dimensions	a = 7.7429(6) Å	$\alpha = 90°$
	b = 21.3255(13) Å	$\beta = 108.494(10)°$
	c = 10.8055(9) Å	$\gamma = 90°$
Volume	1692.1(2) Å^3	
Z	4	
Density (calculated)	1.544 mg/m^3	
Absorption coefficient	0.534 × mm^{-1}	
F(000)	808	
Crystal size	0.3 × 0.3 × 0.3 mm^3	
θ-Range for data collection	2.20–25.00°	
Index ranges	$-8 \leqslant h \leqslant 8$, $-25 \leqslant k \leqslant 25$, $-12 \leqslant l \leqslant 12$	
Reflections collected	21849	
Independent reflections	2849 [R(int) = 0.0522]	
Completeness to θ = 25.00°	95.7%	
Absorption correction	None	
Refinement method	Full-matrix least-squares on F^2	
Data/restraints/parameters	2849/0/230	
Goodness-of-fit on F^2	1.190	
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.0491, wR_2 = 0.1486	
R indices (all data)	R_1 = 0.0563, wR_2 = 0.1524	
Largest diff. peak and hole	0.587 and −0.265 e.Å^{-3}	

5.3.6. Ethyl-3-amino-6,7-dichloro-4-cyano-5-acetoxy-benzo[*b*]furan-2-carboxylate 11a

To a stirred suspension of 500 mg (1.59 mmol) ethyl 3-amino-benzo[*b*]furan-2-carboxylate **9a** in 2.5 ml acetic anhydride was added 0.05 ml DBU. The mixture was stirred for 2 h. The precipitation was isolated and washed with Diethylether. 74 mg (13%), mp 183 °C, (white needles). IR 2231, 1788, 1676. ^{1}H NMR ($CDCl_3$) δ 5.40 (s, br, 2H, –NH_2), 4.54 (q, J = 7.0 Hz, 2H, $COOCH_2CH_3$), 2.56 (s, 1H, CH_3CO) 1.52 (t, J = 7.0 Hz, 3H, $COOCH_2CH_3$). ^{13}C NMR (50 MHz, DMSO-d_6) 171.5 (q, J = 6.2 Hz, CO-acetyl), 159.5 (t, J = 3.1 Hz, CO-ester), 153.8 (s, C-5), 143.1 (s, C-7a), 136.9 (s, C-6), 127.1 (s, C-3a), 123.2 (s, C-7), 122.4 (s, –CN), 119.4 (s, C-2), 113.7 (s, C-3), 90.2 (s, C-4), 60.4 (t, $COOCH_2CH_3$), 20.6 (q, OCOCH3), 14.0 (q, OCH_2CH_3). MS 355 (24, M$^+$), 313 (100), 267 (24), 241 (12). Anal. Calcd for $C_{14}H_{10}Cl_2N_2O_5$: (357.15): C, 47.08; H, 2.82; N, 7.84. Found: C, 47.25; H, 2.80; N, 7.74.

5.3.7. 5-Acetoxy-3-amino-6,7-dichlor-4-cyano-2-(3-methyl-but-2-enoyl)-benzofu[*b*]furan 11s

400 mg (1,23 mmol) **9s** (synthesized according to **9a** starting from **2s** and DDQ **5a**) and 2 ml acetic anhydride, one drop pyridine, 1 h stirred at room temperature. The precipitation is filtered and recrystallized from toluene/acetone. 95 mg (21%), mp 224 °C, yellow needles. IR 3490, 3351, 2231, 1780, 1649, ^{1}H NMR ($CDCl_3$) δ 6.76 (s, 1H), 5.91 (s, 2H, NH_2), 2.48 (s, 3H, CH_3CO), 2.34 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), MS 366 (10, M$^+$), 325 (25), 324 (100), 281 (45), 241 (15), 186 (9), 158 (8), 55 (55). Anal. Calcd for $C_{16}H_{12}Cl_2N_2O_4$ (367,19): C, 52.34; H, 3.29; N, 7.63. found: C, 52.13; H, 3,18; N, 7.50.

5.4. Ethyl-3-amino-4-aminomethylen-5-hydroxy-benzo[*b*]furan-2-carboxylate–HCl 10

2.0 g (6.4 mmol) **9a** and 0.3 g Pd/C (10%) in 70 ml methanol are shaken for 4 h under normal pressure in a H_2 atmosphere. Concentration in vacuo after filtration yields 495 mg (27%), mp 198 °C (white powder from methanol). IR 1673. ^{1}H NMR (DMSO-d_6) δ 10.32 (s, br s, 1H, OH), 8.32 (s, br s, 3H, –$CH_2NH_3^+$), 7.44 (d, J = 9.1 Hz, 1H, H-6), 7.20 (d, J = 9.1 Hz, 1H, H-7), 5.98 (s, 2H, –NH_2-3), 4.53–4.08 (m, 4H, CH_2-4 and $COOCH_2CH_3$), 1.31 (t, 3H, $COOCH_2CH_3$). MS 250 (33, M$^+$-base), 233 (46). Anal. Calcd for $C_{12}H_{14}N_2O_4$–HCl (286,7): C, 50.27; H, 5.27; N, 9.77. Found: C, 49.19; H, 5.03; N, 9.34.

5.5. Ethyl-3-acetylamino-4-acetylaminomethylen-5-acetoxy-benzo[*b*]furan-2-carboxylate 12

350 mg Ethyl 3-amino-4-aminomethylen-5-hydroxy-benzo[*b*]-furan-2-carboxylate–HCl **10** (1.18 mmol), 2 ml acetic anhydride, 0.05 ml pyridine, 40 mg (9%), mp 104 °C, (grey powder from acetic anhydride). IR 1762, 1716, 1688, 1647. ^{1}H NMR ($CDCl_3$) δ 9.39 (s, 1H, –NH-3), 7.57 (d, ^3J = 9.1 Hz, 1H, H-6), 7.20 (d, J = 9.1 Hz, 1H, H-7), 6.36 (t, 1H, –$CH_2NHCOCH_3$), 4.67 (d, J = 7.4 Hz, 2H, –$CH_2NHCOCH_3$), 4.49 (q, J = 7 Hz, 2H, $COOCH_2CH_3$), 2.47 (s, 3H, $OCOCH_3$), 2.38 (s, 3H, –$NHCOCH_3$), 2.05 (s, 3H, $CH_2NHCOCH_3$), 1.49 (t, J = 7 Hz, $COOCH_2CH_3$). ^{13}C NMR (50 MHz, $CDCl_3$) 170.6, 170.4, 170.1, 159.4, 151.6, 146.1, 139.0, 125.0, 123.9, 123.5, 122.9, 113.2, 61.7, 33.8, 23.6, 23.3, 21.0, 14.3. MS 376 (4, M$^+$), 334 (15), 291 (75), 249 (100), 203 (73), 161 (23), 119 (14). Anal. Calcd for $C_{18}H_{20}N_2O_7$ (376,37 × 0.5 CH_3COOH) C, 56.15; H, 5.46; N, 6.89. Found: C, 55.84; H, 4.91; N, 6.98.

5.6. 5-Acetoxy-3-amino-6,7-dichlor-4-cyano-2-(5-morpholino-4-yl-5-oxo-pentanoyl)-benzo[*b*]furan 14

180 mg (1 mmol) **13** and 272 mg (1.2 mmol) DDQ **5a** are stirred at 85 °C for 4 h. The solvent is removed under reduced pressure and treated with 3.5 ml acetic acid anhydride and one drop of pyridine. After some hours the precipitation is filtered and washed with diethylether to yield 115 mg (25%), mp 178 °C. IR 3471, 3361, 2233, 1782, 1651. ^{1}H NMR ($CDCl_3$) δ 5.87 (s, 2H, NH_2), 3.71–3.50 (m, 8H, CH_2–morpholin), 3.10 (t, 2H), 2.61–2.43 (m, 5H, $CH_2 + CH_3CO$), 2.18 (m, 2H, CH_2). ^{13}C NMR (50 MHz, $CDCl_3$) 192.4, 171.5, 167.7, 147.7, 146.8, 137.3, 136.6, 128.9, 124.7, 120.2, 112.9, 98.8, 67.3, 67.1, 46.4, 42.4, 38.1, 32.6, 20.7, 19.4. MS 467 (19, M$^{+\cdot}$), 425 (14), 382 (38), 338 (100), 284 (68), 269 (42), 213 (22), 129 (54), 55 (96). Anal. Calcd for $C_{20}H_{19}Cl_2N_3O_6$ (468,30) C, 51.30; H, 4.09; N, 8.97. Found: C, 50.89; H, 4.29; N, 8.79.

5.7. 1-(5-Hydroxy-benzofuran-3-yl)-4-methyl-pent-2-en-1-on 15

0,2 g (1,85 mol) *p*-Benzoquinone and 0,28 g (1,83 mol) **2s** are stirred at room temperature in 3,5 ml glacial acetic acid. The precipitation is filtered to yield 150 mg (38%), mp 141 °C, yellow powder. IR 3273, 1644. ^{1}H NMR (DMSO-d_6) 9.41 (s, 1H, OH), 8.91 (s, 1H, 2-H), 7.56 (d,1H, 4-H, J = 2.5 Hz), 7.45 (d, 1H, 7-H, J = 9.1 Hz), 6.77–6.85 (m, 2H, 6-H, butenon-H), 2.20 (s, 3H, CH_3), 1.97 (s, 1H, CH_3). MS 216 (92, M^+), 173 (18), 161 (33), 134 (10), 105 (16), 77 (13), 54 (43). Anal. Calcd for $C_{13}H_{12}O_3$ (216,24): C, 72.21; H, 5.59. Found: C, 71.70 H, 5.61.

5.8. CK2 inhibition testing

Recently, we reported on a new capillary electrophoreses (CE) assay for testing inhibition of human proteinkinase CK2.[13] This assay was applied for testing the spirocompounds and benzofuran derivatives on enzyme inhibition. In brief, recombinant purified CK2 holoenzyme (0.25 μg) was mixed with buffer 1 (50 mM Tris/HCl, pH 7.4, 100 mM NaCl, 10 mM MgCl2, 1 mM DTT) in a total volume of 80 μL. Buffer 2 consisted of 25 mM Tris/HCl, pH 8.5, supplemented with 150 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, 100 μM ATP and 190 μM substrate peptide (RRRDDDSDDD-EDANS). Enzyme reaction was started by adding 120 μL buffer 2 to 80 μL of CK2-supplemented buffer 1. For inhibitor testing, 80 μL of the CK2-supplemented buffer 1 was either pre-incubated with 2 μL test compound, previously dissolved in pure DMSO, or incubated with pure DMSO as a control, for 10 min at 37 °C before it was mixed with 120 μL buffer 2. The mixture was incubated for 15 min at 37 °C and the reaction was stopped by the addition of 2 μmol EDTA. Samples were stored at 4 °C until they were injected into the capillary. Bare fused silica capillaries (Beckman Coulter GmbH, Krefeld, Germany) with 50 cm effective and 60 cm total length (50 μm ID and 375 μm OD) were used in a ProteomeLab PA800 System (Beckman Coulter GmbH, Krefeld, Germany). 2 mol/L aqueous acetic acid was used as background electrolyte and UV detection was performed at 214 nm. Before use the capillary was conditioned with 0.1 mol/L NaOH, rinsed with deionized water and equilibrated with 2 mol/L acetic acid prior to sample injection. This procedure was repeated after to every measurement. For IC_{50} value determination of a compound, nine different concentrations ranging from 0.01 μM to 100 μM were tested. One hundred percent CK2 activity was determined in the absence of the compound but in the presence of DMSO. A control value for 0% enzyme activity was obtained by adding all components but without the co-substrate ATP. CK2 activity of a sample including the test compound was calculated as a fraction of the CK2 activity, measured without test compound but with DMSO (positive control).

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References and notes

1. Christian Asche, *Ph. D. Thesis*, Heinrich-Heine-Universität: Düsseldorf, 2002.; (b) Asche, C.; Frank, W.; Albert, A.; Kuckländer, U. *Bioorg. Med. Chem.* **2005**, *13*, 819.
2. Schenck, L. W.; Sippel, A.; Kuna, K.; Frank, W.; Albert, A.; Kuckaender, U. *Tetrahedron* **2005**, *61*, 9129.
3. Becker, H.-D. Quinones as oxidants and dehydrogenating agents. In *The Chemistry of Quinonoid Compounds*; Patai, S., Ed., Part 1; John Wiley & Sons: London, 1974. Chapter 7.
4. Tanemura, K.; Suzuki, T.; Horaguchi, T. *Bull. Chem. Soc. Jpn.* **1993**, *66*, 1235.
5. Bhattacharya, A.; DiMichele, L. M.; Dolling, U. H.; Grabowski, E. J. J.; Grenada, V. J. *J. Org. Chem.* **1989**, *54*, 6118.
6. Ricardo Bollig, *Ph.D. Thesis*, Heinrich-Heine-University: Duesseldorf, 2007.
7. Weiss, M. T.; Allen, G. R.; Gibbs, G.; Piddacks, C.; Poletto, J. F.; Remers, W. A. In *Topics in Hetrocyclic Chemistry*; Castle, R. N., Ed.; Wiley–Interscience: New York, 1965.
8. Kuckländer, U. *Liebigs Ann. Chem.* **1978**, 140.
9. Monks, A.; Scudiero, D.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A. *J. Natl. Cancer Inst.* **1991**, *83*, 757.
10. Boyd, M. R.; Paull, K. D. *Drug Dev. Res.* **1995**, *34*, 91.
11. Guerra; Issinger, O. G. *Curr. Med. Chem.* **2008**, *15*, 1870.
12. Pallares, J.; Llobet, D.; Santacana, M.; Eritja, N.; Velasco, A.; Cuevas, D.; Lopez, S.; Palomar-Asenjo, V.; Yeramian, A.; Dolcet, X.; Matias-Guiu, X. *Am. J. Pathol.* **2009**, *174*, 287.
13. Gratz, A. C.; Götz, J. J. *Electrophoresis* **2010**, *31*, 634.
14. CCDC-776775 (**8a**) and CCDC-776774 (**9a** × DMSO) contain the supplementary crystallographic data (excluding structure factors) for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
15. Sheldrick, G. M.; SHELXS86. *Program for the Solution of Crystal Structures*, University of Göttingen: Germany, 1985.
16. Sheldrick, G. M.; SHELXL97. *Program for the Refinement of Crystal Structures*, University of Göttingen: Germany, 1997.