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Synthesis of new analogs of benzotriazole, benzimidazole and phthalimide—potential inhibitors of human protein kinase CK2

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ABSTRACT

New derivatives of 4,5,6,7-tetrabromo-1*H*-1,2,3-benzotriazole (TBBt), 4,5,6,7-tetrabromo-1*H*-benzimidazole (TBBi), and *N*-substituted tetrabromophthalimides were synthesized and their effect on the activity of human protein kinase CK2 was examined. The most active were derivatives with *N*-hydroxypropyl substituents (IC_{50} in 0.32–0.54 μM range) whereas derivatives of phthalimide were almost ineffective.

1. Introduction

Protein phosphorylation, the most prevalent post-translational process in living cells, is catalyzed by protein kinases. The first protein kinase activity described in 1954 by Burnett and Kennedy[1] was found in rat liver mitochondria using casein as a substrate, hence the enzymes were later called casein kinase 1 (CK1) and casein kinase 2 (CK2). Subsequently, more than 500 protein kinases were discovered and their role in the control of nearly all aspects of cell life and death has been studied widely. It is now recognized that protein kinases are often functionally interlinked and highly regulated, forming a complex communicative network.[2] Abnormal phosphorylation of proteins mediated by kinases may result in diseases, which include cancer, diabetes, rheumatoid arthritis and hypertension.

Protein kinase CK2 is an evolutionarily conserved serine/threonine kinase of tetrameric form, composed of two catalytic α (and/or α′) subunits and two regulatory β subunits that form native structures exhibiting the stoichiometry: $\alpha_2\beta_2$, $\alpha'_2\beta_2$ and/or $\alpha\alpha'\beta_2$. Another catalytic isoform—CK2α″ and its proapoptotic function was reported in human cells.[3] There is strong evidence supporting functional specialization of CK2 catalytic subunits; CK2α and CK2α′ show cell cycle-dependent differences in subcellular localization, as well as differences in phosphorylation pattern and in interaction with protein partners.

CK2 may function as a sensor of cell integrity due to antiapoptotic function caused by its ability to phosphorylate proteins that would be designated for caspase mediated degradation during apoptosis.[4] Inhibitors of CK2 trigger apoptosis and increase the susceptibility of cancer cells to chemotherapeutic agents.[5,6] The increased expression of CK2 is observed in response to various growth stimuli, and its activity is aberrantly elevated in various tumor types, like breast carcinoma, adenocarcinoma of the lung,[7] prostate carcinoma[8] and lymphomas. Genetic studies in yeast[9] indicate that CK2 is required for progression through both G1/S and G2/M transitions. In mammalian cells, forced expression of kinase-inactive CK2α or CK2α′ subunits compromises cell proliferation.[10] Because inhibition of CK2 has a potential role in the treatment of breast and other cancers, therefore the major efforts are currently devoted to the development of specific CK2 inhibitors.

Several classes of CK2 inhibitors, effective in low micromolar ranges, have been reported. The specific inhibitors of CK2, 4,5,6,7-tetrabromo-1*H*-1,2,3-benzotriazole[11] (TBBt), 4,5,6,7-tetrabromo-1*H*-benzimidazole (TBBi) and 4,5,6,7-tetrabromo-1*H*-benzimidazole-2-N,N-dimethylamine (DMAT)[12] are used very widely to elucidate the role of CK2 phosphorylation of a variety of proteins with important metabolic functions.[13–15] Bearing in mind the role of CK2 in regulation of cell proliferation and the fact that TBBt, TBBi and DMAT can induce apoptosis,[16,17] we undertook modification of TBBt and TBBi in order to obtain more potent inhibitors of CK2.

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The basis for TBBt and TBBi selectivity is provided by the hydrophobic pocket adjacent to the ATP/GTP binding site which is smaller in CK2, than in the majority of other protein kinases. Structural information regarding the CK2α subunit in complex with various inhibitors[18,19] indicates conformational plasticity around the ATP binding site. Since TBBt and derivatives of TBBi may have an effect on the conformation of the Gly-rich loop at the active site of CK2, it seemed interesting to explore the effect of alkyl substituents with different hydrophobic, steric and electrophilic features attached to TBBt and TBBi on their inhibitory potency. Another group of compounds that can fit into the small ATP binding pocket could be derivatives of phthalimide. Phthalimide rings are present in numerous biologically active compounds. For example, naphthalimides such as amonafide, dendric imides with *N*-polyamine tails were synthesized and studied as antitumor agents,[20] whereas 5′-*N*-phthaloyl-3′-azido-2′,3′-dideoxythymidine derivatives were evaluated for their antiviral activity against HIV-1, HIV-2 and Moloney murine sarcoma virus. Compound with the tetrabromophthaloyl moiety inhibited both HIV-1 and HIV-2 in the low micromolar range.[21] Thalidomide, as well as simple halogenated phthalimides have shown bidirectional TNF-α production regulating properties.[22]

We designed new derivatives of TBBt, TBBi and phthalimide and evaluated their effect on human CK2 alfa and human holoenzyme activities.

2. Material and methods

2.1. Chemistry

The starting materials—4,5,6,7-tetrabromo-1*H*-benzimidazole (TBBi) and 4,5,6,7-tetrabromo-1*H*-benzotriazole (TBBt) were synthesized by bromination of 1*H*-benzimidazole[23] or 1*H*-benzotriazole[24] according to published methods. The *N*-alkyl derivatives (**4, 5, 10, 11**, **18**) were synthesized by alkylation of TBBt or TBBi with the use of alkyl halides in the presence of DBU, NaH or KOH as bases (Scheme 1).

N-Hydroxyalkyl derivatives (**2, 3**, **7**, **8**, **17**) were prepared by alkylation of TBBt or TBBi with the use of NaH, KOH or DBU as bases and appropriate hydroxyalkyl halide as reported in Refs. 25,26. The ratio of N^1 to N^2 substituted anomers of TBBt depends on the reaction conditions and on the length of the alkyl chain. For the synthesis of compounds **14** and **15** a different strategy was used. The hydroxyl group of 4-bromobutan-1-ol was protected with 3,4-dihydro-2*H*-pyran before using it in the alkylation reaction. The yield of **14** was very low, so that compound was not used for biological tests.

Compound **6** was obtained by reaction of dimethylamine with derivative **5** (Scheme 2). The phosphorylation of compound **8** with the use of $POCl_3$ and subsequent purification gave derivative **9**. Derivatives **12** and **13** were obtained by reaction of 1-methylpiperazine with **10** and **11** respectively.

N-Substituted tetrabromophthalimides (**19–21**) were obtained by reaction of tetrabromophthalic anhydride with different amines. The latter compound in reaction with hydrazine gave 5,6,7,8-tetrabromo-2,3-dihydrophthalazine-1,4-dione (**22**) (Scheme 3).

2.2. Expression of subunits of CK2 and determination of CK2 activity

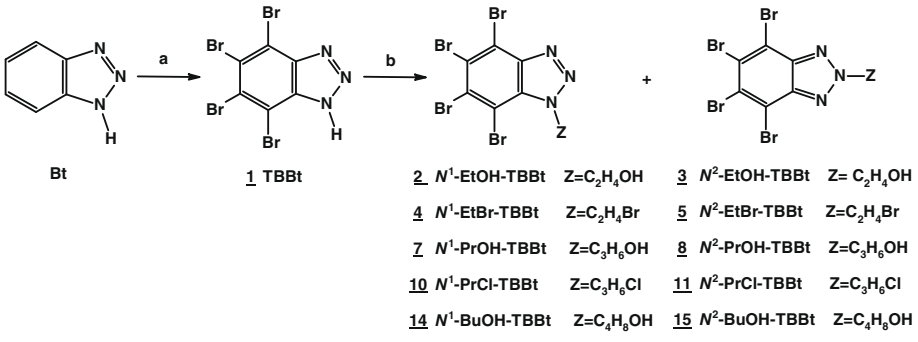
The pT7-7 vector carrying human CK2α subunit (hCK2α) cDNA and the pT7-7 vector carrying human CK2β subunit (hCK2β) cDNA were a kind gift of Dr. Stefania Sarno, University of Padova, Italy. Expression and purification of rhCK2a and rhCK2b proteins was done according to published methods.[27,28]

The reaction mixture (final volume of 50 μL) for the determination of CK2 activity (apoenzyme CK2α or holoenzyme CK2α2β2) contained: casein substrate (75 μg) or peptide substrate (RRRDDDSDDD, Biosyntan, Germany, 20 μM), Tris–HCl pH 7.5 (20 mM), $MgCl_2$ (20 mM), γ[^{32}P]ATP (10 μM, 100–200 cpm pmol^{-1}) and appropriate concentrations of inhibitor in 1 μL DMSO. After 20 min of incubation at 30 °C, 40 μL of the assay mixture was spotted onto a square (2 cm × 2 cm) of Whatman 3 MM (for casein) or P81 (for peptide) paper, which was immediately immersed in cold 5% (w/v) trichloroacetic acid containing 0.3% *o*-phosphoric acid (10 mL per square), and washed with H_2O five times for 10 min. Then the squares were washed in 96% ethanol and allowed to dry. The radioactivity was quantified using a LKB Flexi-Vial liquid scintillation counter.

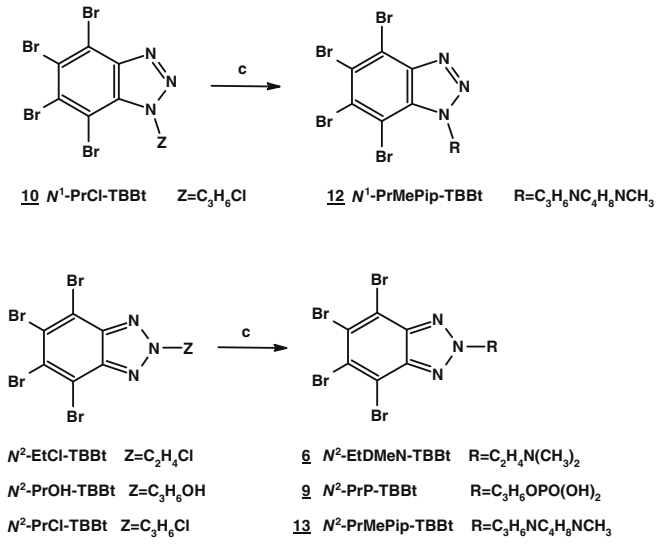
The activity was calculated as the percentage of incorporated ^{32}P (measured in scintillation counter) in the presence of various concentrations of the studied compounds versus control with appropriate concentration of DMSO. Microcal™Origin Version: 6.0 and Excel (Microsoft) were used to calculate the data.

3. Results and discussion

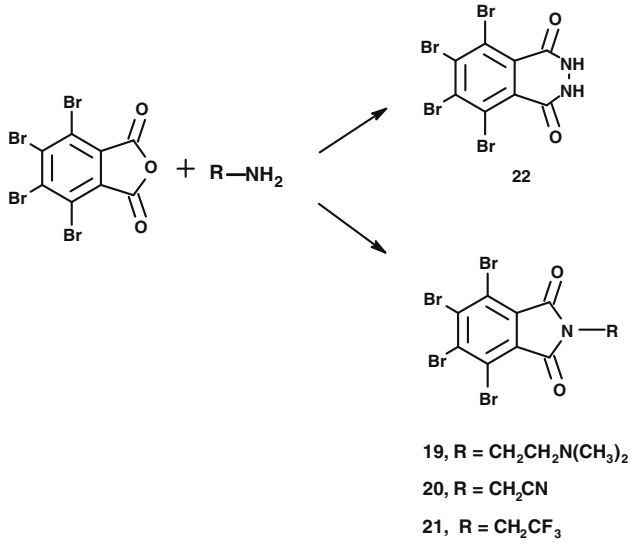
Our search for new inhibitors of protein kinase CK2 was based on the knowledge of the inhibitory activity of benzimidazole and benzotriazole derivatives. The four atoms of bromine in the benzene ring seem to be an essential requirement for biological activity. Another substituents in the benzene ring, four atoms of chlorine or four methyl groups, leads to significantly lower inhibitory effect versus *Saccharomyces cerevisiae*[23], rat and recombinant human CK2. Thus, we focused on TBBt and TBBi as the leading compounds used for modification. We compared the effect of N^1 versus N^2-alkyl derivatives of TBBt on their inhibitory potency. Additionally, we examined the effect of the length of the alkyl substituent on CK2 activity.



Scheme 1. Synthesis of derivatives of 4,5,6,7-tetrabromo-1*H*-benzotriazole: (a) Br_2, HNO_3; (b) **2** and **3**: $Br(CH_2)_2OH$, DBU, CH_3CN, reflux, 12 h; **4** and **5**: $Br(CH_2)_2Br$, KOH in CH_3CN, 60 °C, 12 h; **7** and **8**: $Br(CH_2)_3OH$, DBU, CH_3CN, RT; **10** and **11**: $Cl(CH_2)_3Br$, NaH in dry DMF, rt; **14** and **15**: 4-chloro-1-(2-tetrahydropyranyloxy)-butane, NaH, DMF, *p*-toluenesulfonic acid, rt, 48 h.



Scheme 2. Reagents and conditions (c) **6** $(CH_3)_2NH$/EtOH, 70 °C, 24 h; **9** TMP, $POCl_3$, 0 °C to rt; **12** and **13** $C_4H_9N_2CH_3$, K_2CO_3, CH_3CN, 70 °C.



Scheme 3. Synthesis of derivatives of tetrabromophthalic anhydride.

The influence of the synthesized compounds on human CK2α subunit and on the holoenzyme activity was determined as described in Materials and Methods. The results are shown in Table 1. For compounds which exerted a small effect on the activity of CK2α, the inhibition of holoenzyme was not determined. When IC_{50} was much higher than 10 μM, the CK2α activity at the presence of 5 μM concentration of the tested compound was evaluated.

Some of the new analogs showed IC_{50} similar to that of the parent compounds (**1** and **16**) and their inhibitory activity depended on the length of the alkyl substituents. The 3-(4,5,6,7-tetrabromo-1*H*-benzimidazol-1-yl)propan-1-ol (**17**) exhibited a higher potency (IC_{50} 0.54 ± 0.15 μM) than the parent compound TBBi (IC_{50} 1.30 ± 0.13 μM). The 3-(4,5,6,7-tetrabromo-1*H*-1,2,3-benzotriazol-1-yl)propan-1-ol (**7**) and 3-(4,5,6,7-tetrabromo-2*H*-1,2,3-benzotriazol-2-yl)propan-1-ol (**8**) also exhibited higher potency against the catalytic subunit of CK2α that TBBt (**1**), with IC_{50} 0.32 ± 0.15 μM and 0.34 ± 0.12 μM compared to 0.50 ± 0.07 μM for **1**. Derivatives with shorter (**3, 4, 5**) or longer chains (**12, 13, 15**) showed lower inhibitory activity. The activity differed slightly with a change of triazole on the imidazole ring. The small differences between N^1 versus N^2 substituents may prove the plasticity of the binding pocket of CK2.

The change of triazole ring in the phthalimide moiety (compounds **19–21**) or phthalazine (**22**) results in loss of inhibitory potency.

The attempt to correlate the inhibitory activity of the synthesized compounds with theirs lipophilicity was undertaken (Table 1). When Clog*P* of derivatives is compared, it can be seen that for lower Clog*P* values the inhibitory potency is higher, albeit no strict relationship can be observed. The difference between Clog*P* of known inhibitor DMAT and compound **6** (4.74 vs 5.14) may partially explain the difference in inhibitory potency of those two compounds, albeit this is not observed for compounds **2** or **12** with low Clog P (4.25 and 4.63, respectively) and low inhibitory potency (9.25 μM and 11.20 μM vs CK2α).

4. Conclusions

In the present study we report the evaluation of new TBBt, TBBi and phthalimide derivatives as inhibitors of CK2 protein kinase.

To improve the inhibitory activity versus CK2, TBBt and TBBi were subjected to chemical modifications. Most modifications either partially or completely abrogated the ability of the new derivatives to inhibit CK2. However, selected modifications with the hydroxypropyl substituent resulted in compounds with comparable or better inhibitory activity than the parent compounds. In particular, 3-(4,5,6,7-tetrabromo-1*H*-1,2,3-benzotriazol-1-yl)propan-1-ol (**7**), 3-(4,5,6,7-tetrabromo-2*H*-1,2,3-benzotriazol-2-yl)propan-1-ol (**8**), and 3-(4,5,6,7-tetrabromo-1*H*-benzimidazol-1-yl)propan-1-ol (**17**) showed a small improvement in CK2 inhibitory activity.

5. Experimental

Melting points were determined in open capillary tubes using Büchi apparatus B504 and are uncorrected. UV absorption spectra were recorded on a Cary 300 spectrophotometer. Thin-layer chromatography (TLC) was performed on 0.2 mm Merck silica gel 60 F_{254} plates. Preparative separations were carried out by column chromatography using silica gel (230–400 mesh), or PLC silica gel 60 F_{254} 2 mm glass plates from Merck. ^1H and ^{13}C NMR spectra were recorded on Varian 500, 400 or 200 MHz spectrometer at 298 K. Chemical shifts (δ) are reported in parts per million units (ppm) relative to tetramethylsilane (Me_4Si, $\delta = 0$ ppm). Mass spectrometry was recorded on Micro-mass ESI Q-TOF spectrometer at IBB PAN.

Starting materials: 1*H*-benzimidazole and 1*H*-1,2,3-benzotriazole, were purchased from Aldrich Co; 1*H*-1,2,3-benzotriazole and 1*H*-benzimidazole derivatives were synthesized adapting the methods developed in our laboratory: 4,5,6,7-tetrabromo-1*H*-1,2,3-benzotriazole (**1**, TBBt),[24] 2-(4,5,6,7-tetrabromo-1*H*-1,2,3-benzotriazol-1-yl)ethanol (**2**, N^1-EtOH-TBBt) and 2-(4,5,6,7-tetrabromo-2*H*-1,2,3-benzotriazol-2-yl)ethanol (**3**, N^2-EtOH-TBBt) as reported in Ref. 25, 4,5,6,7-tetrabromo-1*H*-benzimidazole (**16**, TBBi) as published in Ref. 23, 3-(4,5,6,7-tetrabromo-1*H*-1,2,3-benzotriazol-1-yl)propan-1-ol (**7**, N^1-PrOH-TBBt), 3-(4,5,6,7-tetrabromo-2*H*-1,2,3-benzotriazol-2-yl)propan-1-ol (**8**, N^2-PrOH-TBBt) and 3-(4,5,6,7-tetrabromo-1*H*-benzimidazol-1-yl)propan-1-ol (**17**, *N*-PrOH-TBBi) were synthesized as reported in patent application P 380112. The Clog*P* values were obtained using ChemDraw Pro 8.0 software.

5.1. 4,5,6,7-Tetrabromo-1-(2-bromoethyl)-1*H*-1,2,3-benzotriazole (4, N^1-EtBr-TBBt) and 4,5,6,7-tetrabromo-2-(2-bromoethyl)-2*H*-1,2,3-benzotriazole (5, N^2-EtBr-TBBt)

To the solution of TBBt (200 mg, 0.46 mmol) in acetonitrile (2.5 mL) the solution of KOH (112 mg, 2 mmol) in MeOH (1 mL) was added, followed by 1,2-dibromoethane (1.6 mL), and the reac-

Table 1
Inhibition of the CK2 protein kinase activity by derivatives of 1*H*-benzotriazole, 1*H*-benzimidazole and phthalimide

	Compound	CK2α activity[a] (%)	IC_{50}[μM] CK2α	IC_{50}[μM] $CK2\alpha_2\beta_2$	*C*log *P*
1	TBBt		0.50 ± 0,07	0.5 ± 0.12	4.53
2	N^1-EtOH-TBBt		9.25 ± 1.09	2.61 ± 0.72	4.25
4	N^1-EtBr-TBBt	60			5.6
5	N^2-EtBr-TBBt	79			5.97
6	N^2-EtDiMetN-TBBt	75			5.14
7	N^1-PrOH-TBBt		0.32 ± 0.15	0.48 ± 0.14	4.36
8	N^2-PrOH-TBBt		0.34 ± 0.12	0.82 ± 0.19	4.73
9	N^2-PrP-TBBt		2.2 ± 0.22	2.6 ± 0.17	5.27
10	N^1-PrCl-TBBt	87		5.87 ± 2.24	5.58
11	N^2-PrCl-TBBt	69			5.96
12	N^1-PrMePip-TBBt		11.20 ± 4.84	2.50 ± 0.59	4.63
13	N^2-PrMePip-TBBt		7.3 ± 4.89	8.6 ± 3.41	5.0
15	N^2-BuOH-TBBt		1.73 ± 0.15	1.52 ± 0,30	5.19
16	TBBi		1.30 ± 0.23	0.93 ± 0.08	4.13
17	*N*-PrOH-TBBi		0.54 ± 0.15	0.71 ± 0.04	3.95
18	*N*-PrCl-TBBi		2.9 ± 0.20	1.1 ± 0.14	5.18
19	*N*-EtdMeN-TBiI	88			4.3
20	*N*-MeCN-TBiI	80			4.05
21	*N*-tFEt-TBiI	88			5.28
22	TBPht	95			3.58

[a] CK2α activity at the presence of 5 μM concentration of the tested compound.

tion mixture was heated at 60 °C for 12 h. The same amount of KOH and 1,2-dibromoethane were added twice again to complete the reaction. The resulting precipitate was filtered, washed with water, dissolved in chloroform and the products were separated by chromatography with the use of CH_3Cl:hexane 1:1. After elution and evaporation the products **4** (42 mg, 16.8% yield) and **5** (54 mg, 21.7% yield) were obtained.

5.2. (4, N^1-EtBr-TBBt)

Mp 198–199.5 °C, R_f 0.39 ($CHCl_3$:hexane 1:1); 0.89 ($CHCl_3$:MeOH 98:2). UV (MeOH + 4% CH_3CN) λ_{max} (ε) 277 (8400), 288 (8300), 310 (5500), 321 (3800); ^{1}H NMR (Me_2SO-d_6) δ: 4.03 (t, 2H, 2′-CH_2), 5.35 (t, 2H, 1′-CH_2); MS $[M+H]^+$ *m/z* calcd for $C_8H_5Br_5N_3^+$, 541.6359, found, 541.6775.

5.3. (5, N^2-EtBr-TBBt)

Mp 163.7–164.7 °C, R_f 0.63 ($CHCl_3$:hexane 1:1); 0.9 ($CHCl_3$:MeOH 98:2). UV (MeOH + 7% CH_3CN) λ_{max} (ε) 284 (11,000), 298 (14,400), 307 (15,000), 321 (7000) ^{1}H NMR (Me_2SO-d_6) δ: 4.14 (t, 2H, 2′-CH_2), 5.27 (t, 2H, 1′-CH_2); MS $[M+H]^+$ *m/z* calcd for $C_8H_5Br_5N_3^+$, 541.6359, found 541.6575.

5.4. *N*,*N*-Dimethyl-*N*-[2-(4,5,6,7-tetrabromo-2*H*-1,2,3-benzotriazol-2-yl)ethyl]amine (6, N^2-EtDMeN-TBBt)

The mixture of 4,5,6,7-tetrabromo-2-(2-chloroethyl)-2*H*-1,2,3-benzotriazole[25] (200 mg, 0.40 mmol) and ethanolic dimethylamine (12 mL, 20%) was heated in steel autoclave at 70 °C for 20 h. After cooling the resulting precipitate was crystallized from MeOH/dioxane to obtain **6**, (126 mg, 60% yield) mp 125–126 °C, R_f 0.035 ($CHCl_3$:hexane 1:1); UV (MeOH + 25% CH_3CN) λ_{max} (ε) 284 (10,000), 297 (12,000), 306 (12,600); ^{1}H NMR (Me_2SO-d_6) δ: 2.18 (s, 6H, 2 × CH_3), 2.95 (t, 2H, CH_2), 4.91 (t, 2H, CH_2); ^{13}C NMR (Me_2SO-d_6) δ: 45.6, 55.7, 58.5, 114.3, 126.2, 143.2; EI ASC 506.8 $[M+H]^+$, 528.8 $[M+Na]^+$; MS $[M+H]^+$ *m/z* calcd for $C_{10}H_{11}Br_4N_4^+$ 506.7676, found 506.8237.

5.5. 3-(4,5,6,7-Tetrabromo-1*H*-1,2,3-benzotriazol-1-yl)propan-1-ol (7, N^1-PrOH-TBBt)

Mp 180–182 °C, R_f 0.65 ($CHCl_3$:MeOH 9:1); UV: pH 7 λ_{max} (ε) 281 (8500), 291 (8900), 311 (6300); MeOH λ_{max} (ε) 278 (8700), 289 nm (8600), 308 nm (6000); ^{1}H NMR (Me_2SO-d_6) δ: 2.06–2.11 (qui, 2H, 2′-CH_2); 3.50 (q, 2H, 3′-CH_2); 4.64 (t, 1H, OH); 4.98 (t, 2H, 1′-CH_2); ^{13}C NMR (Me_2SO-d_6) δ: 33.72; 47.54; 57.45; 106.70; 115.62; 123.34; 128.33; 131.67; 144.84. MS $[M+H]^+$ *m/z* calcd for $C_9H_8Br_4N_3O^+$ 493.7360, found 493.7782.

5.6. 3-(4,5,6,7-Tetrabromo-2*H*-1,2,3-benzotriazol-2-yl)propan-1-ol (8, N^2-PrOH-TBBt)

Mp 139.6–139.9 °C; R_f 0.71 ($CHCl_3$:MeOH 9:1), UV pH 7 λ_{max} (ε): 300 (12,800), 307 (13,400); MeOH λ_{max} (ε): 297 (13,000), 306 (13,500); ^{1}H NMR (Me_2SO-d_6) δ: 2.17–2.22 (qui, 2H, 2′-CH_2); 3.49 (q, 2H, 3′-CH_2); 4.72 (t, 1H, OH); 4.86 (t, 2H, 1′-CH_2); ^{13}C NMR (Me_2SO-d_6) δ: 32.38; 54.28; 57.37; 113.47; 125.36; 142.41. MS $[M+H]^+$ *m/z* calcd for $C_9H_8Br_4N_3O^+$ 493.7360, found 493.7855.

5.7. 3-(4,5,6,7-Tetrabromo-2*H*-1,2,3-benzotriazol-2-yl)propyl dihydrogen phosphate (9, N^2-PrP-TBBt)

A solution of N^2-PrOHTBBt (200 mg, 0.4 mmol) and $POCl_3$, (0.300 mL) in trimethylphosphate (1.6 mL) was cooled to 0 °C for 30 min., stirred at rt for a few hours, and neutralized with $NaHCO_3$. The product was separated with the use of DEAE–Sephadex, with a gradient of TEAB buffer. After the evaporation and lyophylisation compound **9**, (N^2-PrP-TBBt) was obtained. R_f 0.54 (*i*-prop:NH_3:H_2O 7:1:2); UV (MeOH) λ_{max} 283, 296, 306; ^{1}H NMR (D_2O) δ: 2.47 (m, 2H, 2′-CH_2), 3.98 (dt, 2H, 3′-CH_2), 4.95 (t, 2H, 1′-CH_2), ^{31}P NMR (D_2O) δ: 2.32; MS $[M-H]^-$ *m/z* calcd for $C_9H_7Br_4N_3O_4P^-$ 571.6867, found 571.6853.

5.8. 4,5,6,7-Tetrabromo-1-(3-chloropropyl)-1*H*-1,2,3-benzotriazole (10, N^1-PrCl-TBBt) and 4,5,6,7-tetrabromo-2-(3-chloropropyl)-2*H*-1,2,3-benzotriazole (11, N^2-PrCl-TBBt)

To a suspension of 60% sodium hydride (36 mg, 0.9 mmol) in DMF (4.5 mL) cooled to 0 °C TBBt (200 mg, 0.46 mmol) was added and the mixture was stirred for 30 min. To this solution 1-bromo-3-chloropropan (0.500 mL) was added and the reaction was stirred overnight at reflux. The solution was poured into cold water, the resulting precipitate was filtered off, dissolved in $CHCl_3$ and products were separated with the use of PLC ($CHCl_3$:hexane 1:1). After elution and evaporation compounds **10** (37 mg, 15.8% yield) and **11** (142 mg, 60% yield) were obtained.

Compound **10**: mp 170.0–171.2 °C; R_f 0.40 ($CHCl_3$:hexane 1:1), 0.89 ($CHCl_3$:MeOH 98:2); ^{1}H NMR (Me_2SO-d_6) δ: 2.41 (qui, 2H, 2′-

CH_2), 3.71 (t, 2H, 3′-CH_2), 5.06 (t, 2H, 1′-CH_2). ^{13}C chemical shifts from ^{13}C HSQC and HMBC (Me_2SO-d_6) δ: 33.16 (2′-CH_2), 41.94 (3′-CH_2), 47.59 (1′-CH_2), 132.02 (7a-C); MS $[M+H]^+$ *m/z* calcd for $C_9H_7Br_4ClN_3^+$ 511.7021, found 5.1182.

Compound **11**: mp 160–162 °C, R_f 0.75 ($CHCl_3$:hexane 1:1), 0.93 ($CHCl_3$:MeOH 98:2) UV (MeOH + 14% dioxane) λ_{max} (ε) 270 (7600), 277 (8500), 288 (8000), 310 (5200) ^1H NMR (Me_2SO-d_6) δ: 2.58 (qui, 2H, 2′-CH_2), 3.69 (t, 2H, 3′-CH_2), 4.95 (t, 2H, 1′-CH_2) MS $[M+H]^+$ *m/z* calcd for $C_9H_7Br_4ClN_3^+$ 511.7021, found 511.7951.

5.9. 4,5,6,7-Tetrabromo-1-[3-(4-methylpiperazin-1-yl)propyl]-1*H*-1,2,3-benzotriazole (12, N^1-PrMePip-TBBt)

To the mixture of 4,5,6,7-tetrabromo-1-(3-chloropropyl)-1*H*-1,2,3-benzotriazole (40 mg, 0.078 mmol) and K_2CO_3 (40 mg), in 1.5 mL of acetonitrile, N^1-methyl piperazine (0.052 mL, 0.469 mmol) was added and heated at 70 °C for 72 h. After cooling, the reaction mixture was evaporated and the products were separated by PLC with the use of $CHCl_3$:MeOH 9:1. After the elution and evaporation compound **12** was obtained (10 mg, 22.3% yield). ^1H NMR (Me_2SO-d_6) δ: 1.90–2.20 (br s and br m; 13H, 2′-CH_2 and piperazine), 2.34 (t, 2H, 3′-CH_2), 4.99 (t, 2H, 1′-CH_2); ^1H NMR (Me_2SO-d_6, *t* = 323 K) δ: 1.92–2.00 (br s, 4H, piperazine), 2.02 (s, 3H, CH_3), 2.08 (m, 2H, 2′-CH_2), 2.14–2.18 (br s, 4H, piperazine), 2.35 (t, 2H, 3′-CH_2), 4.99 (t, 2H, 1′-CH_2); MS $[M+H]^+$ *m/z* calcd for $C_{14}H_{18}Br_4N_5^+$ 575.8255, found 575.7589.

5.10. 4,5,6,7-Tetrabromo-2-[3-(4-methylpiperazin-1-yl)propyl]-2*H*-1,2,3-benzotriazole (13, N^2-PrMePip-TBBt)

To the mixture of 4,5,6,7-tetrabromo-2-(3-chloropropyl)-2*H*-benzotriazole (160 mg, 0.312 mmol) and K_2CO_3 (140 mg) in acetonitrile (4 mL), 1-methylpiperazine (0.200 mL, 1.8 mmol) was added and heated to reflux for 48 h. After cooling, the reaction mixture was partitioned between water and chloroform. The organic layer was concentrated and the products were separated by PLC with the use of $CHCl_3$:MeOH 98:2. After the elution and evaporation compound **13** was obtained (80 mg, 44.5% yield). Mp 97–99 °C, R_f 0.04 ($CHCl_3$:MeOH 98:2); UV: (MeOH) 284 (9000), 298 (11,400), 306.5 (11,700); ^1H NMR (Me_2SO-d_6) δ: 1.90–2.40 (br s, br m, br t; 15H, 2′-CH_2, 3′-CH_2 and piperazine), 4.83 (t, 1′-CH_2); MS $[M+H]^+$ *m/z* calcd for $C_{14}H_{18}Br_4N_5^+$ 575.8255, found 575.7624.

5.11. 4-(4,5,6,7-Tetrabromo-1*H*-1,2,3-benzotriazol-1-yl)butan-1-ol (14, N^1-BuOH-TBBt) and 4-(4,5,6,7-tetrabromo-2*H*-1,2,3-benzotriazol-2-yl)butan-1-ol (15, N^2-BuOH-TBBt)

4-Chlorobutanol-1 (0.45 mL) in ether (4.6 mL) containing two drops of concd HCl was stirred and cooled to 0 °C. 3,4-Dihydro-2*H*-pyran (0.63 mL) was added and the solution was stirred at room temperature overnight. The solution was neutralized with $NaHCO_3$ and extracted with ether. The organic layer was washed with water and brine, dried (Na_2SO_4) and evaporated. After evaporation 846 mg of 4-chloro-1-(2-tetrahydropyranyloxy)-butane as an oil was obtained.

To the mixture of TBBt (200 mg, 0.46 mmol) in DMF (1.5 mL) with 60% sodium hydride (24 mg, 0.6 mmol), K_2CO_3 (33 mg) was added and the mixture was stirred and heated to 100 °C for 30 min. The solution of 4-chloro-1-(2-tetrahydropyranyloxy)-butane (280 mg) in DMF (1 mL) was added and the reaction mixture was heated overnight. After cooling, the precipitate was filtered off; the filtrate was evaporated, the residue was diluted with chloroform, extracted with water and the organic layer was evaporated. *p*-Toluenesulfonic acid (150 mg) in ethanol (4 mL) was added to the residue and stirred for 48 h at room temperature. The solvent was removed under reduced pressure, residue poured into dichloromethane, washed with 2 N NaOH, and water, dried with Na_2SO_4, and products were separated with the use of SiO_2 plates (ethyl acetate:methanol 20:1). After elution and evaporation 4-(4,5,6,7-tetrabromo-1*H*-benzotriazol-1-yl)butan-1-ol (**14**, 3 mg, 1.28% yield) and 4-(4,5,6,7-tetrabromo-2*H*-benzotriazol-2-yl)butan-1-ol (**15**, 125 mg, 53.6%) were obtained.

Compound **14**: R_f 0.017 ($CHCl_3$:hexane 1:1); 0.49 ($CHCl_3$: MeOH 98:2), MS $[M+H]^+$ *m/z* calcd for $C_{10}H_{10}Br_4N_3O^+$ 507.7516, found 507.7692.

Compound **15**: mp 179–179.9 °C, R_f 0.03 ($CHCl_3$:hexane 1:1); 0.59 ($CHCl_3$ MeOH 98:2) UV (MeOH + 17% CH_3CN) 284 (10,700), 297 (13,700), 306 (14,300), 320 (6600). ^1H NMR (Me_2SO-d_6) δ: 1.45 (qui, 2H, 3′-CH_2), 2.07 (qui, 2H, 2′-CH_2), 3.43 (q, 2H, 4′-CH_2), 4.49 (t, 1H, OH), 4.82 (t, 2H, 1′-CH_2). ^{13}C chemical shifts from ^{13}C HSQC and HMBC: (Me_2SO-d_6) δ: 25.92 (2′-CH_2), 29.81 (3′-CH_2), 57.00 (1′-CH_2), 59.70 (4′-CH_2). MS $[M+H]^+$ *m/z* calcd for $C_{10}H_{10}Br_4N_3O^+$ 507.7516, found 507.8037.

5.12. 3-(4,5,6,7-Tetrabromo-1*H*-benzimidazol-1-yl)propan-1-ol; (17, *N*-PrOH-TBBi)

Mp 196.4–197.2 °C, R_f 0.41 ($CHCl_3$:MeOH 9:1), UV: pH 7 (9% dioxane) λ_{max} (ε) 269 nm (9700), 274 nm (9650), 301 nm (3800); ^1H NMR (Me_2SO-d_6) δ: 1.93–1.98 (qui, 2H, 2′-CH_2); 3.41 (q, 2H, 3′-CH_2); 4.57 (t, 2H, 1′-CH_2); 4.68 (t, 1H, OH); 8.46 (s, 1H, 2-CH); ^{13}C NMR: 34.21; 43.81; 57.23; 106.49; 116.43; 120.26; 122.24; 131.28; 143.60; 148.94. MS $[M+H^+]$ *m/z* calcd for $C_{10}H_9Br_4N_2O^+$ 492.7407, found 492.7268.

5.13. 4,5,6,7-Tetrabromo-1-(3-chloropropyl)-1*H*-benzimidazole (18, *N*-PrCl-TBBi)

Obtained as for **10**, purified by chromatography ($CHCl_3$:MeOH 99:1) 125 mg, (53% yield) mp 141–142 °C, R_f 0.73 ($CHCl_3$:MeOH 98:2); UV (MeOH + 10%CH_3CN) λ_{max} (ε): 268 (10,400), 273 (10,350), 302 (3750); ^1H NMR (Me_2SO-d_6) δ: 2.29 (m, 2H, 2′-CH_2); 3.69 (t, 2H, 3′-CH_2), 4.63 (t, 2H, 1′-CH_2), 8.49 (s, 1H, 2-CH); MS $[M+H]^+$ *m/z* calcd for $C_{10}H_8Br_4ClN_2^+$ 510.7069, found 510.7082.

5.14. General procedure for *N*-substituted tetrabromophthalimides

4,5,6,7-Tetrabromophthalic anhydride (10 mmol) and proper amine (10 mmol) were dissolved in dry DMF (15 mL) and refluxed for 2 h. Then the mixtures were cooled and poured into water. Crude products were filtered and crystallized from 90% ethanol.

5.15. 4,5,6,7-Tetrabromo-2-[2-(dimethylamino)ethyl]-1*H*-isoindole-1,3(2*H*)-dione (19, *N*-EtDMeN-TBiI)

Mp 225 °C, UV (MeOH) λ_{max} (ε) 244 (28,350), 335 (1400).^1H NMR (Me_2SO-d_6) δ: 4.05 (t, 2H, *J* = 6.9 Hz), 2.99 (t, 2H, *J* = 6.9 Hz), 2.02 (s, 6H, 2 × CH_3). IR (KBr, cm^{-1}): 1776, 1726 (C=O). Anal. Calcd for $C_{12}H_{10}Br_4N_2O_2$: C, 27.00; H, 1.89; N, 5.25. Found: C, 27.04; H, 1.94, N, 5.22.

5.16. (4,5,6,7-Tetrabromo-1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)acetonitrile (20, *N*-MeCN-TBiI)

Mp 305 °C, UV (MeOH) λ_{max} (ε) 246 (31,000), 340 (2500). ^1H NMR (Me_2SO-d_6) δ: 4.58 (s, 2H, CH_2). IR (KBr, cm^{-1}): 2228 (–CN), 1778, 1725 (C=O). Anal. Calcd for $C_{10}H_2Br_4N_2O_2$: C, 23.94; H, 0.40; N, 5.58. Found: C, 23.90; H, 0.42; N, 5.55.

5.17. 4,5,6,7-Tetrabromo-2-(2,2,2-trifluoroethyl)-1*H*-isoindole-1,3(2*H*)-dione (21, N-tFEt-TBiI)

Mp 213 °C, UV (MeOH), λ_{max} (ε) 246 (33,000), 338 (2300). ^{1}H NMR (Me_2SO-d_6) δ: 3.96 (q, J = 13 Hz, CH_2). IR (KBr, cm^{-1}): 1777, 1726 (C═O). Anal. Calcd for $C_{10}H_2Br_4F_3NO_2$: C, 22.05; H, 0.37; N, 2.57. Found: C, 22.06; H, 0.39; N, 2.60.

5.18. 5,6,7,8-Tetrabromo-2,3-dihydrophthalazine-1,4-dione (22, TBPht)

Tetrabromophthal-anhydride (10 mmol) in dry ethanol (20 mL) were refluxed over 30 min. with hydrazine monohydrate (15 mmol). Then 10 mL of 10% HCl was added and heated during next 30 min. The crude product was filtered and crystallized from dioxane; mp 299 °C UV (MeOH) λ_{max} (ε) 254 (25,500), 330 (2900). ^{1}H NMR (Me_2SO-d_6) δ: 4.22 (s, 2H, 2 × CH) IR (KBr, cm^{-1}): 1740 (C═O). Anal. Calcd for $C_8H_2Br_4N_2O_2$: C, 20.11; H, 0.42; N, 5.86. Found: C, 20.06; H, 0.39; N, 5.90.

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