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Chemistry of polyhalogenated nitrobutadienes, 19: Synthesis of new types of compounds modulating the biological activity of Type I Interferons (IFN-I)

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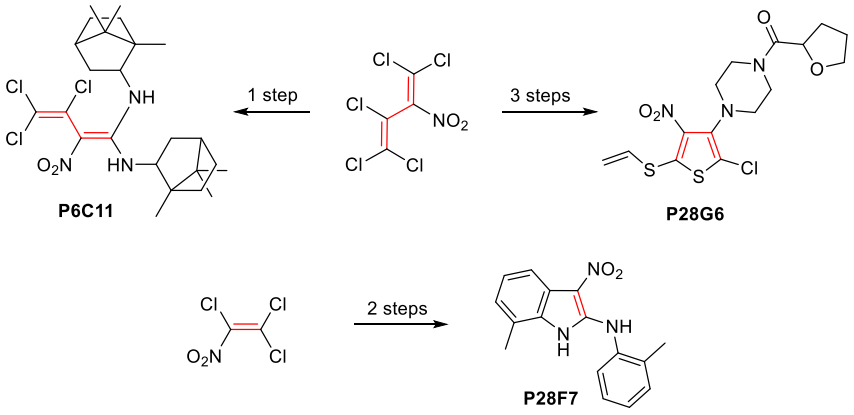
Abstract

The synthesis of ten new types of compound derived from polyhalonitroalkenes is described starting from the versatile solvent trichloroethene. The new compounds modulate the biological activity of Type I Interferons (IFN-I). Three of them (bornylamine derivative **P6C11**, chlorothiophene **P28G6**, and nitroindole **P28F7**) are promising candidates to inhibit rhIFN activity.



Keywords: Nucleophilic vinylic substitution, 2-nitropentachlorobutadiene, heterocyclizations, indoles, pyrazoles, thiophenes, Interferons

Introduction

Halogenated nitrobutadienes are a relatively small subsection in the group of nitroalkene compounds.[1] Halonitrobutadienes are easily accessible by introduction of an activating and directing nitro group into polyhalo-1,3-butadienes,[2] which can be obtained by dimerization of versatile solvents such as trichloroethene and 1,2-dichloroethenes, followed by subsequent dehydrohalogenation-halogenation reactions. Polyhalonitrobutadienes are valuable precursors for highly functionalized acyclic or (hetero)cyclic compounds.[1,3] Especially perchloro-2-nitrobutadiene (**13**) has often served as a starting material of choice, due to its enhanced reactivity in S_NVin processes.[1,4] Thus, applying selective and mild reaction conditions, this diene enables 'click' chemistry type syntheses.

In previous work we were able to show that certain derivatives of halonitrobutadienes can modulate the biological activity of Type I Interferons (IFN-I).[5] Although IFN-Is have been widely used in the treatment of many viral and malignant diseases,[6] they were also reported to be responsible for the etiopathogenesis of some autoimmune diseases, like systemic lupus erythematosus, Sjögren's syndrome, myositis, dermatomyositis, systemic sclerosis, and insulin-dependent diabetes mellitus. In all of them, IFN-I production is exacerbated and uncontrollable.[7,8] To seek compounds that might be useful to inhibit the IFN-I increase, various analyses have been described in the literature.[9]

In the present paper, we mainly focus on recent progress in the syntheses of acyclic and heterocyclic derivatives of polyhalonitroalkenes that modulate the biological activity of IFN-Is. We have used four polyhalonitroalkenes as starting materials for this work: perchloro-2-nitrobutadiene (**13**), 1-bromotetrachloro-3-nitrobuta-1,3-diene (**6**) as a mixture of two isomers, perchloro-1-nitrobutadiene (**11**) as single isomer (*Z*- or *E*-), and trichloronitroethene (**23**) (Figure 1).

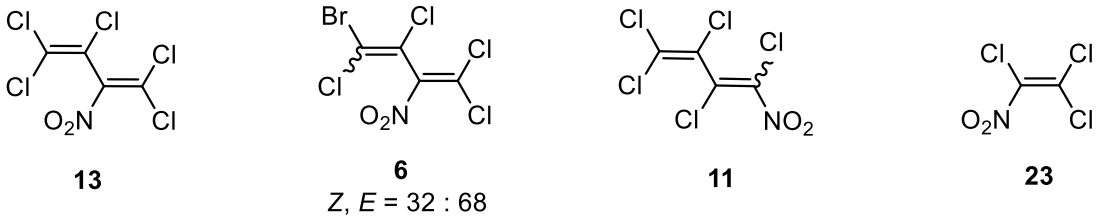


Figure 1. Structures of the four starting compounds.

Results and Discussion

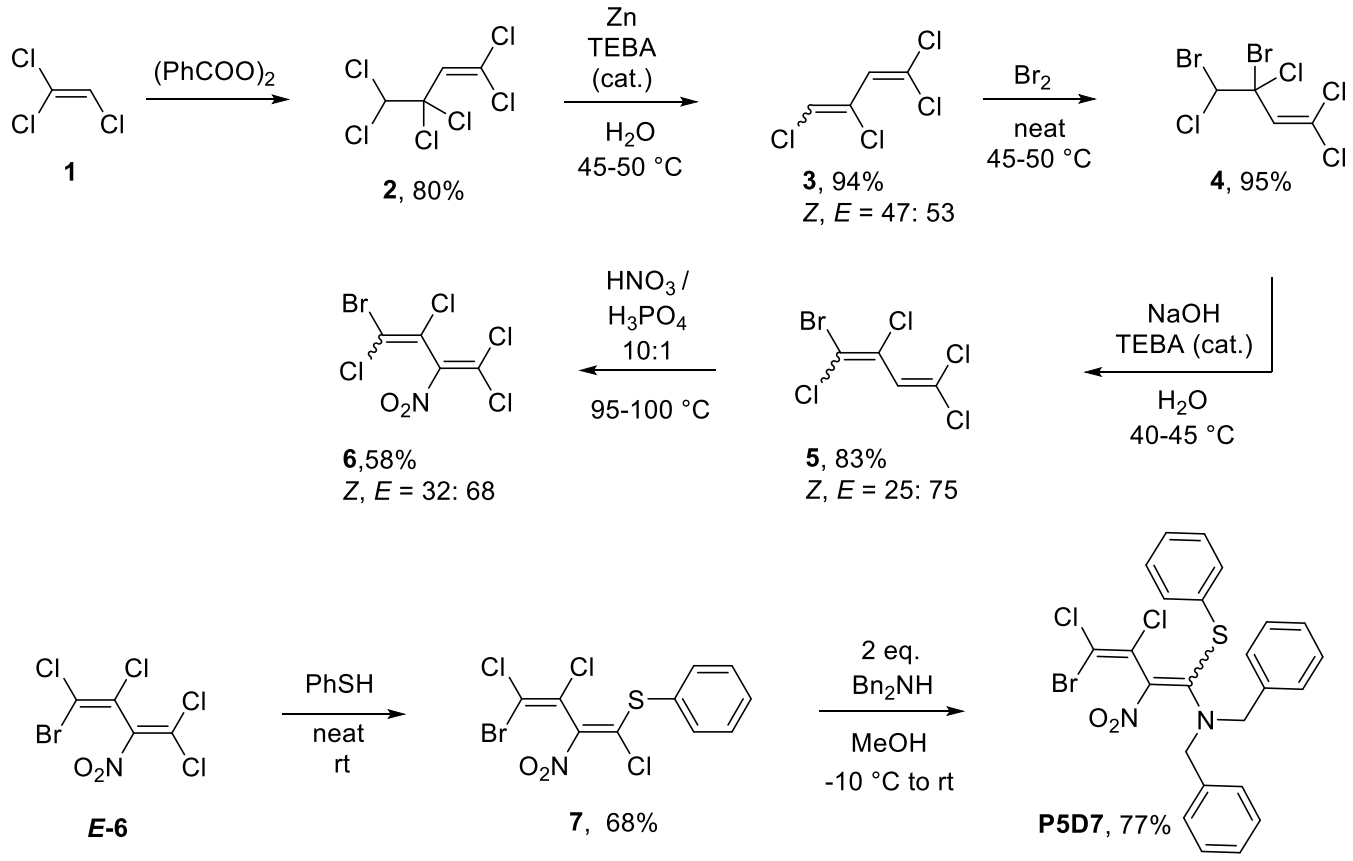
Synthesis

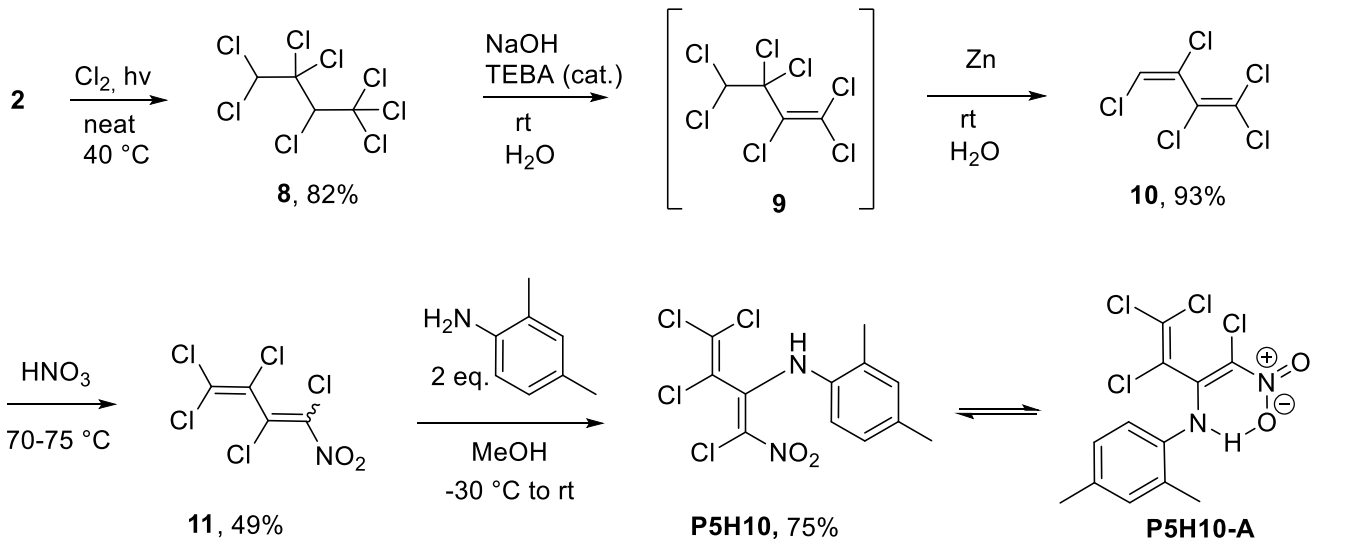
Ten halonitrobutadiene derivatives (**P5D7**, **P5H10**, **P6H1**, **P6C11**, **P28E1**, **P28E9**, **P28F7**, **P28G6**, **P28H3**, and **P28H7**) out of 288 synthetic compounds studied at the Institute of Organic Chemistry, Clausthal University of Technology, Germany, were shown to be highly effective in cell culture systems. The structures of the most active synthetic compounds are depicted in Table 1.

Syntheses of P5D7 and P5H10

Bromonitrodiene P5D7. Dimerization of trichloroethene (**1**) with benzoyl peroxide resulted in butene **2**, the dechlorination of which with zinc dust led to butadiene **3**.[10] Bromination of **3** with subsequent

dehydrobromination of dibromo adduct **4** gave bromodiene **5**. Electrophilic vinylic nitration of **5** provided nitrodiene **6** as a mixture of *Z*-and *E*-isomers (32:68).[11] The crystalline *E*-isomer of **6** reacted with benzenethiol to give the sulfane **7**.[12] Diene **P5D7** as mixture of two isomers (1:1) was readily prepared from **7** *via* nucleophilic vinylic substitution using two equivalents of dibenzylamine.



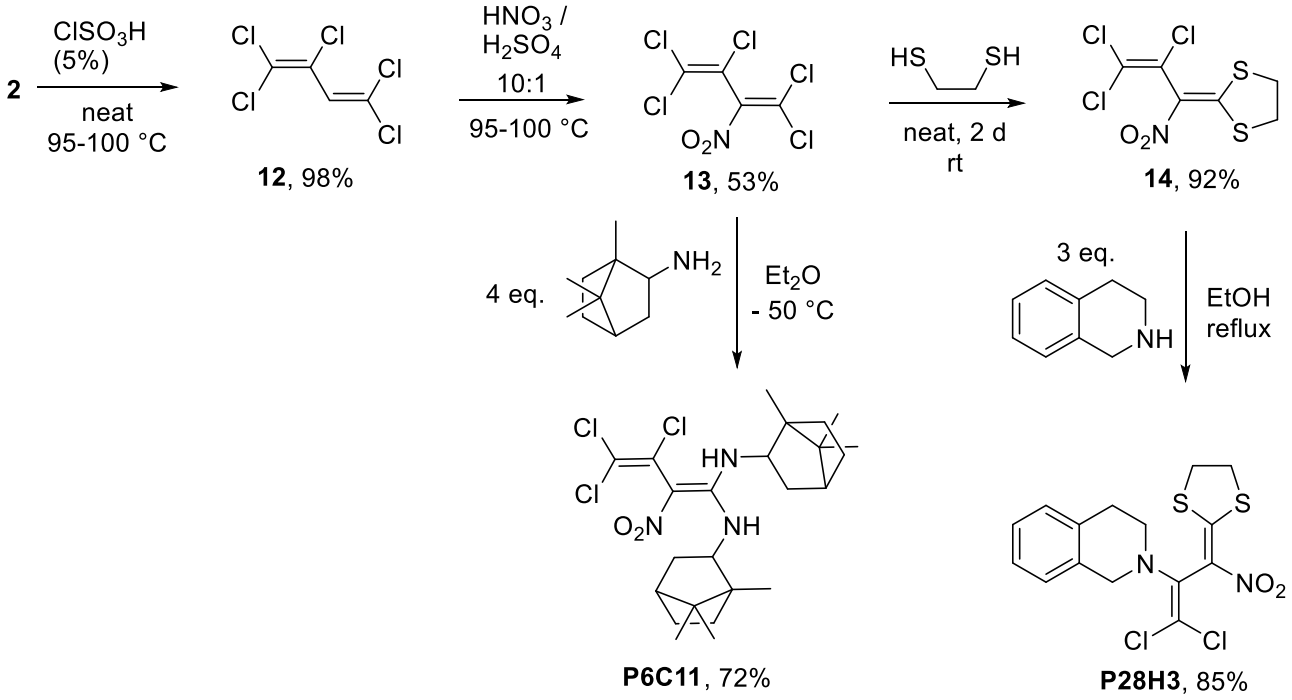


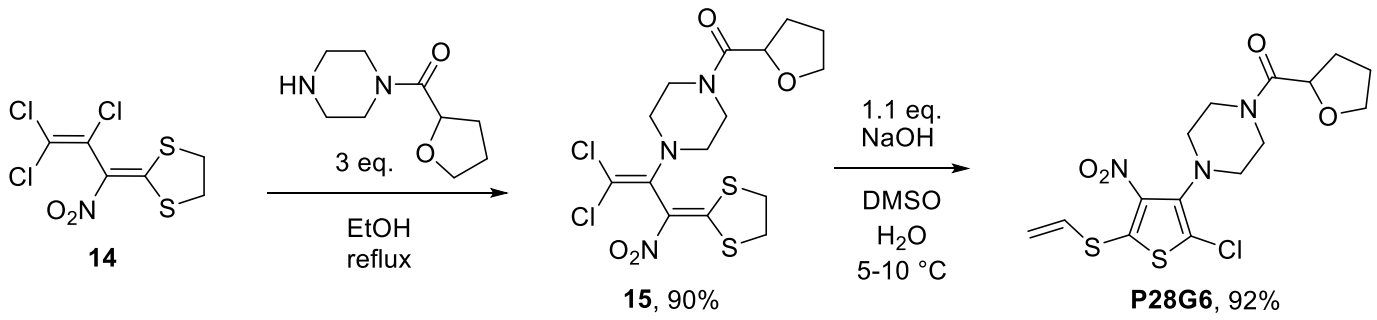
Scheme 1. Synthetic route to bromonitrodiene **P5D7** and 1-nitrobutadiene **P5H10**.

1-Nitrobutadiene P5H10. Chlorination of the dimer of trichloroethene **2** with Cl_2 in the presence of $SbCl_5$ under light gave octachlorobutane **8**.[13] Partial dehydrochlorination of **8** with sodium hydroxide in the presence of a catalytic amount of the phase transfer catalyst benzyltriethylammonium chloride provided heptachlorobutene **9**,[14] which was directly dechlorinated with zinc dust to pentachlorobutadiene **10**.[15] Nitration of diene **10** with nitric acid at 70 – 75 °C led to nitrobutadiene **11**.[16] S_NVin reaction of **11** with 2,4-dimethylaniline in MeOH at -30 °C provided 1-nitrodiene **P5H10** in 75% yield, stabilized through hydrogen bonding between the N–H group and an oxygen of the NO_2 group by formation of a six membered ring **P5H10–A** (Scheme 1). Interestingly, nitrobutadiene **P5H10** has been used in the screening of lifespan-altering compounds of eukaryotic organisms.[17]

Syntheses of P6C11, P28H3, and P28G6

Bornylamine derivative P6C11. Dehydrochlorination of the dimer of trichloroethene **2** with catalytic amounts of chlorosulfonic acid at 95 – 100 °C or with anhydrous iron trichloride at 125 – 130 °C, gave butadiene **12**.[11] Treatment of **12** with nitrating acid at 100 – 105 °C led to nitrobutadiene **13** in 53% yield.[18] Treatment of nitrodiene **13** with four equivalents of bornylamine provided bisaminodiene **P6C11** in 72% yield.[19]





Scheme 2. Synthetic route to bornylamine derivative **P6C11**, dithiolane **P28H3**, and thiophene **P28G6**.

Dithiolane P28H3. Dithiolane **14** was produced solvent free by treatment of nitrodiene **13** with ethane-1,2-dithiol at room temperature.[20] Treatment of **14** with a threefold excess of 1,2,3,4-tetrahydroisoquinoline in refluxing EtOH led to the formation of dithiolane **P28H3** in 85% yield.

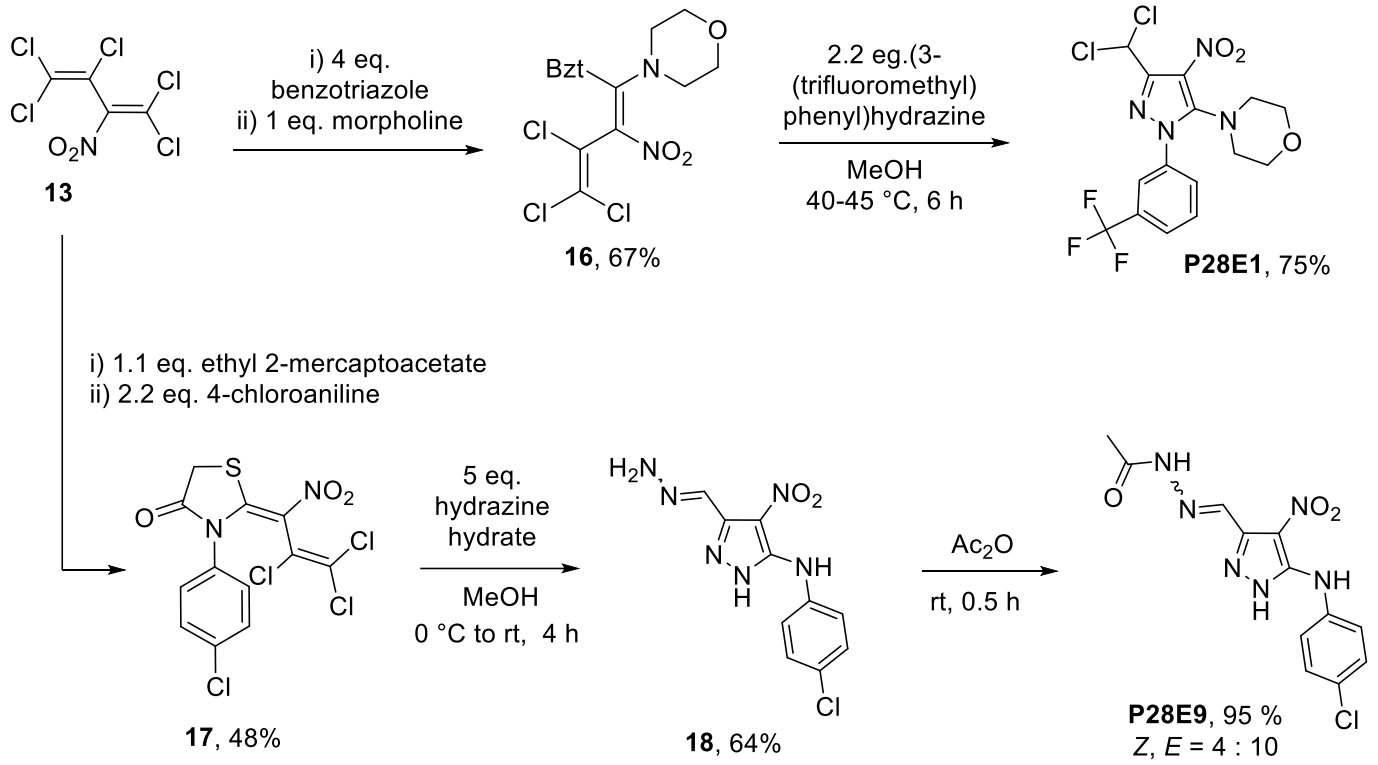
Thiophene P28G6. Compound **14** reacted readily and selectively with a 3-fold excess of piperazin-1-yl(tetrahydrofuran-2-yl)methanone in refluxing EtOH to give dithiolane **15**. The intramolecular cyclization reaction of 2-(2-diorganylamino-3,3-dichloro-1-nitroallylidene)-[1,3]dithiolanes such as **15** upon action of base in DMSO represents a short and efficient domino method for the synthesis of perfunctionalized 3-amino-4-nitrothiophenes. The reaction proceeds via anionic dithiolane ring opening, followed by an S_Ni reaction.[20] The new thiophene **P28G6** was synthesized in this way in 92% yield (Scheme 2).

Syntheses of P28E1 and P28E9

Pyrazole P28E1.

Treatment of nitrodiene **13** with benzotriazole in THF at room temperature led to the formation of 1,1'-(3,4,4-trichloro-2-nitrobuta-1,3-diene-1,1-diyl)bis(1*H*-benzotriazole) in 76% yield.[21] Interaction of this azole with morpholine in methanol at 0 °C to room temperature furnished nitrodiene **16** in 88% yield.[22] Nitrodiene **16** reacted readily and selectively with a 2.2 fold excess of (3-(trifluoromethyl)phenyl)hydrazine in methanol at 40 – 45 °C to give pyrazole **P28E1** in 75% yield.

Pyrazole P28E9. Nucleophilic vinylic substitution of one chlorine-atom in diene **13** with an equimolar amount of ethyl 2-mercaptoacetate without any solvent at room temperature led to the formation of ethyl 2-((1,3,4,4-tetrachloro-2-nitrobuta-1,3-dien-1-yl)thio)acetate in 80% yield. Subsequent amination of this sulfide with a 2.2 fold excess of 4-chloroaniline in methanol at 0 °C to room temperature furnished (*Z*)-3-(4-chlorophenyl)-2-(2,3,3-trichloro-1-nitroallylidene)thiazolidin-4-one (**17**) through cyclization in 60% yield. By treatment of thiazolidinone **17** with a fivefold excess of hydrazine hydrate in methanol at 0 °C to room temperature *N*-(4-chlorophenyl)-3-(hydrazineylidenemethyl)-4-nitro-1*H*-pyrazol-5-amine (**18**) was formed (64% yield) in a one-pot reaction and four reaction steps: formal *ipso*-substitution of the sulfur by hydrazine was followed by hydrolysis of the amide unit and cyclization of the primary hydrazinyl group to form a pyrazole.[23] Additional hydrazine then attacked the *gem*-dichloromethyl group forming hydrazone **18**. Acetylation of **18** with acetic anhydride at room temperature gave pyrazole **P28E9** almost quantitatively, as a mixture of *Z*- and *E*-isomers (4 : 10) (Scheme 3).



Scheme 3. Synthetic route to pyrazoles **P28E1** and **P28E9**.

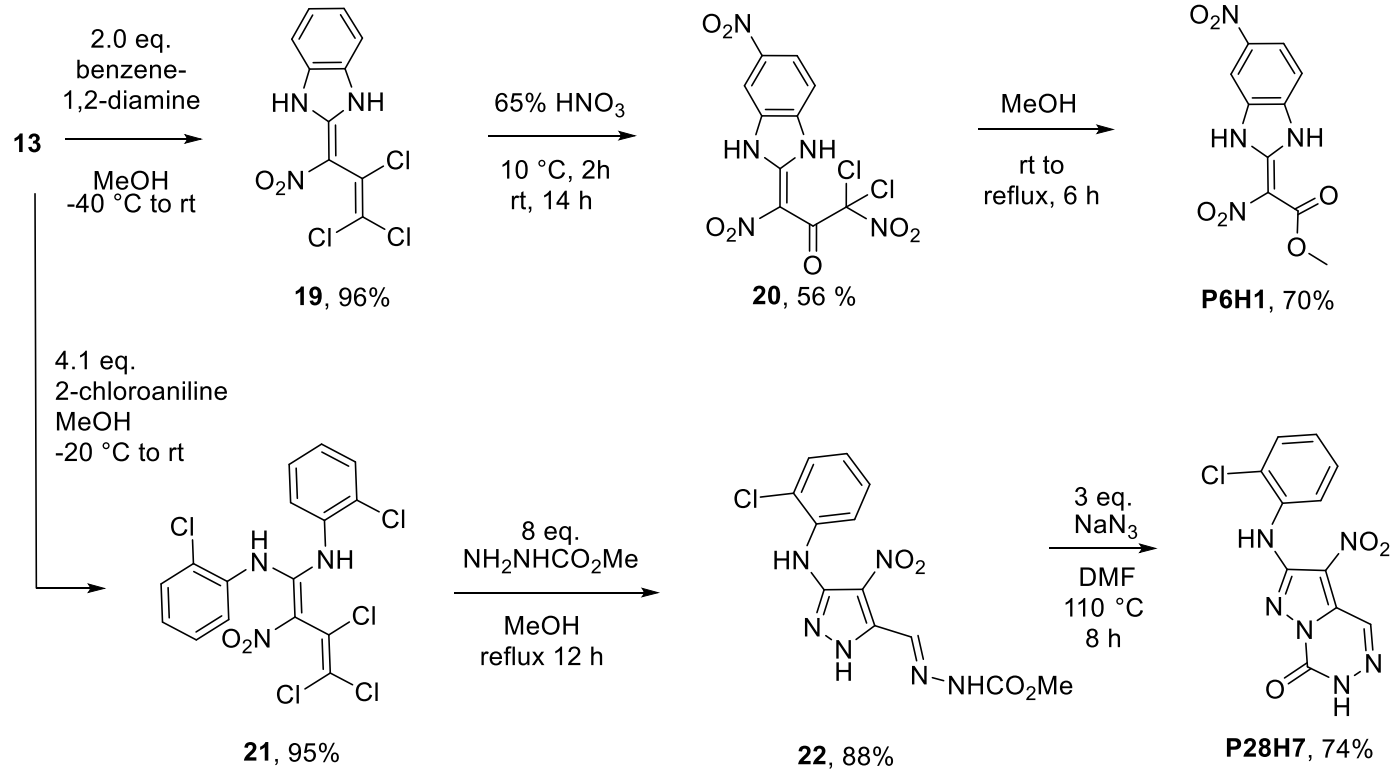
Syntheses of P6H1 and P28H7

Benzimidazole P6H1. Azole **19** was synthesized in 96% yield according to the literature[24] by interaction of diene **13** with a twofold excess of benzene-1,2-diamine in methanol at -40 °C to room temperature. Interestingly, compound **19** was found to act as Clp-protease inhibitor. Clp-protease is known as a target for herbicides or growth regulators.[25] By treatment of compound **19** with 65% nitric acid at 10 °C to room temperature, 1,1-dichloro-1,3-dinitro-3-(5-nitro-1,3-dihydro-2*H*-benzimidazol-2-ylidene)propan-2-one (**20**) was obtained in 56% yield. The assumed mechanism of this unusual transformation can be explained as follows. Firstly, an electrophilic aromatic nitration of the benzene ring in **19** takes place. Next, partial hydrolysis of the trichlorovinylic group with subsequent elimination of HCl from the intermediate chlorohydrin leads to a dichloromethylcarbonyl group. Finally, electrophilic nitration of the dichloronitromethylketone to give **20** takes place. Compound **20** is a water stable solid. The use of stronger nucleophiles such as amines or alcohols opened access to a series of esters and amides. The methyl ester **P6H1** of this series proved to be the best modulator of the biological activity of Type I Interferons. Compound **P6H1** was obtained in 70% yield in the course of a haloform-like reaction by refluxing ketone **20** in MeOH for six hours.

Triazinone P28H7. Butadiene **21** was obtained in 95% yield according to the literature[26] by amination of nitrodiene **13** with a 4.1-fold excess of 2-chloroaniline at -20 °C for 3 hours. The reaction of diene **21** with an 8-fold excess of methyl hydrazinecarboxylate in refluxing methanol for 12 hours les to the formation of pyrazole **22** in 88% yield. The conceivable mechanism for the formation of the compound **22** it the same as for pyrazole **18** and presented in the literature.[23] Heating pyrazole **22** in DMF at 110 °C for 8 hours using three equivalents of sodium azide as base, gave triazinone **P28H7** in 74% yield (Scheme 4). Formally, compound **P28H7** is formed by intramolecular cyclisation of **22**. It is assumed that the process starts with deprotonation of the pyrazolic NH in **22**, followed by intramolecular cyclisation with the methoxycarbonyl group. Finally, thermal elimination of methanol leads to the triazinone **P28H7**. Using other organic or inorganic bases and/or other solvents, formation

of **P28H7** was either not observed or the yield was very low (5-10%). Using additional 1,1-diarylamino-2-nitrotrichlorobutadienes such as **21**, more triazinones similar to **P28H7** were available in two-step syntheses. From this series of triazinones, **P28H7** showed the best effect as modulator of the biological activity of Type I Interferons.

Additional details on the synthetic mechanisms are accessible via the literature citations.



Scheme 4. Synthetic routes to benzimidazole **P6H1** and triazinone **P28H7**.

Triazinones such as **P28H7** are rare in the literature. A SciFinder[27] search of compounds bearing a **P28H7**-skeleton gave only 23 hits (Figure 2). Such pyrazolotriazinones are a new class of human leukocyte elastase inhibitors[28] and can also be used as activator of pro-apoptotic BAX.[29] The BAX gene was the first identified pro-apoptotic member of the Bcl-2 protein family.[30]

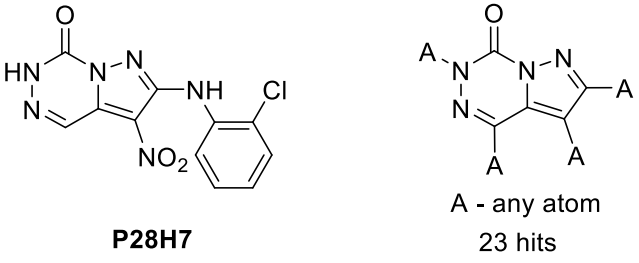
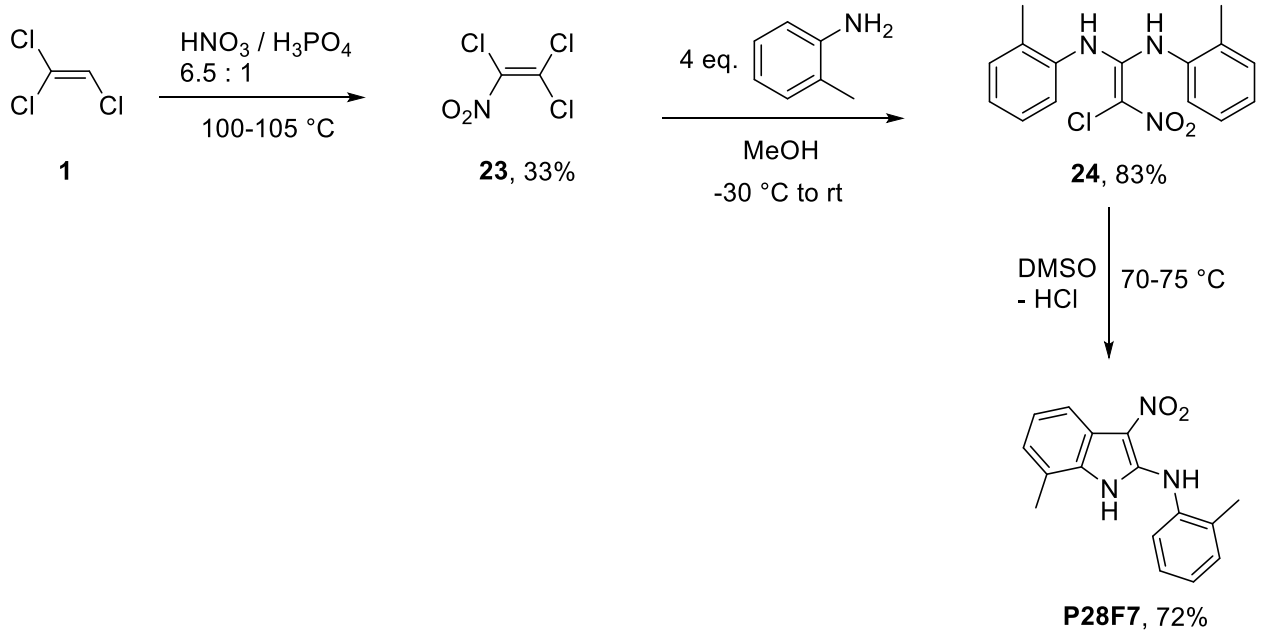


Figure 2. Score of hits of compounds with **P28H7**-skeleton from SciFinder database, accessed on 21.03.2023.

Synthesis of P28F7

Nitroindole P28F7. Nitration of ethene **1** with a mixture of nitric and phosphoric acid (6.5:1) at 100 °C gave nitrotrichloroethene (**23**).[31] Treatment of nitroethene **23** with a fourfold excess of *o*-toluidine at -30 °C led to the formation of endiamine **24** similar to work in reference.[32] Heating **24** in DMSO (in reference[33] ethanol was used as solvent for a similar reaction with aniline) at 70 – 75 °C, nitroindole **P28F7** was produced via an S_Ei reaction in 72% yield (Scheme 5).



Scheme 5. Synthetic route to nitroindole **P28F7**.

Biological activity

Determination of half-maximal inhibitory concentration (IC_{50})

The WISH-Mx2/EGFP reporter cell line[34] was used to determine the IC_{50} value for the synthesized compounds. Cells were seeded into 96-well plates at 2.5 x 10^4 cells/well in 0.1 mL of complete medium consisting of Minimum Essential Medium (MEM) supplemented with 10% (v/v) foetal bovine serum (FBS) and 2 mM of glutamine. They were incubated at 37 °C and 5% CO_2 for 24 h. Supernatants were removed and recombinant human interferon-α2a or -β1a (rhIFN-α2a or rhIFN-β1a) were added at a constant concentration of 40 and 12 IU/mL, respectively, prepared in assay medium (MEM 2% (v/v) FBS and 2 mM glutamine). Immediately thereafter, compounds were added applying two-fold serial dilutions of each one in the assay medium at a concentration range of 14-0.2 µM (each sample was assayed in triplicates). Cells were incubated for 24 h at 37 °C and 5% CO_2. After discarding the supernatants, cells were trypsinized and homogeneously suspended in 0.2 mL of phosphate buffer saline (PBS). Finally, cells were analyzed using a Guava® EasyCyte™ cytometer (Guava Technology, USA). For each sample, 2,000 counts gated on a forward scatter (FSC) vs side scatter (SSC) dot plot excluding cell doublets were recorded. The settings used for the acquisition of fluorescence signal for Enhanced Green Fluorescent Protein (EGFP) were λex/λem=488/530 ± 20. For data acquisition and analysis, the Guava CytoSoft™ 3.6.1 software was employed. Appropriate negative and positive controls were assayed in triplicates. Negative controls were carried out by adding only assay medium while positive controls were accomplished incubating cells with rhIFN-α2a or rhIFN-β1a at 40 or 12 IU/mL, respectively, without adding compounds. The IC_{50} was determined as the

concentration of each compound capable of reducing by half the maximum response achieved by the positive control minus the signal of the negative control.

It is known from the literature that some natural and synthetic compounds can negatively modulate the biological activity of IFN-I. For example, a new antimalarial aminoacridine derivative named X6 showed superior inhibitory action to hydroxychloroquine for the treatment of an experimental autoimmune myocarditis mediated in vivo by the cGAS/stimulator of IFN genes (cGAS/STING) pathway.[35] Another example is thymoquinone that can downregulate IRF-3 activation via inhibition of TBK1, which would subsequently decrease the production of IFN-I.[36] Also, three natural compounds (Vioprolid B, Pellasoren A, and Gephyronic acid A) that inhibit the activity of IFN-I were described by Bürgi et al.[5] In the same publication, the ten synthetic compounds described herein also showed inhibitory actions but their syntheses were not depicted. In this paper, their syntheses are described above and their IC_{50} values determined, precisely.

The IC_{50} is the most widely used parameter to determine drugs' efficacy. It represents the concentration of drug/compound necessary to inhibit a biological process at 50% of the response; in our case, the eGFP percentage as a measurement of IFN-I activity. Considering that the following synthetic compounds (**P5D7, P5H10, P6H1, P6C11, P28E1, P28E9, P28F7, P28G6, P28H3, and P28H7**) showed an inhibitory response on the biological activity of IFN-I,[5] their IC_{50} values were determined using a WISH-Mx2/EGFP cell line-based reporter gene assay (RGA) in the presence of both IFN-I (rhIFN-α2a and rhIFN-β1a). This RGA was previously validated to measure IFN-I potency[33] as well as the effect of certain drugs/compounds to modulate the biological activity of these cytokines.[5,9] The WISH-Mx2/EGFP reporter cell line encompasses the property by which the specific Mx2 promoter drives the EGFP expression after the IFN-I binding to the cell receptor.

The IC_{50} of the halonitrobutadiene derivatives are shown in Table 1. Bornylamine derivative **P6C11**, nitroindole **P28F7**, and chlorothiophene **P28G6** represent the most interesting compounds. They showed the lowest concentrations to inhibit the 50% of the IFN-I activity. Furthermore, these compounds had shown the capacity to revert the IFN activated pathway and have a residual effect to inhibit the IFN activity[5]. These results together with the IC_{50} determination, herein shown, potentiate the use of these compounds as a starting point for the synthesis of molecules with improved properties.

Table 1. Inhibition of IFN-I biological activity by halonitrobutadiene derivatives

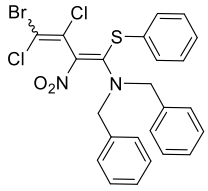
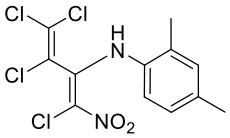
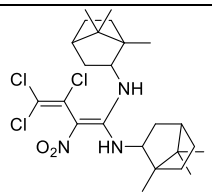
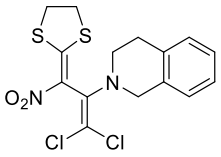
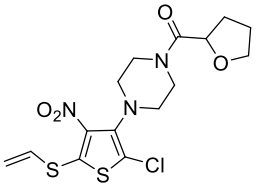
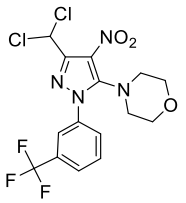
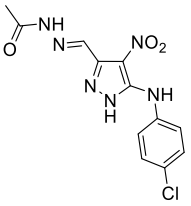
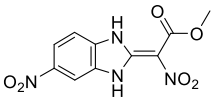
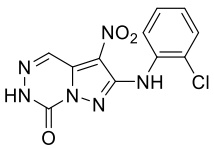
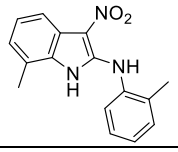
Comp.	Structure	IC_{50} (μM) rhIFN-α2a / rhIFN-β1a
P5D7		8.6 ± 0.1 / 8.12 ± 0.07
P5H10		9.78 ± 0.07 / 10.62 ± 0.05

Table 1. Continued

Comp.	Structure	IC_{50} (μM) rhIFN-α2a / rhIFN-β1a
P6C11		5.63 ± 0.02 / 5.83 ± 0.05
P28H3		10.36 ± 0.04 / 10.53 ±0.04
P28G6		5.30 ± 0.08 / 5.7 ± 0.2
P28E1		11.2 ± 0.1 / 9.67 ± 0.03
P28E9		10.2 ± 0.1 / 9.77 ± 0.01
P6H1		ND
P28H7		10.49 ± 0.08 / 10.32 ± 0.08
P28F7		4.57 ± 0.04 / 5.00 ± 0.07

The IC_{50} was determined as the concentration of compound needed to inhibit 50% of the IFN-I biological activity. ND: non-determined.

Figure 3 summarizes some structures of natural and synthetic compounds that modulate the biological activity of Type I interferons.

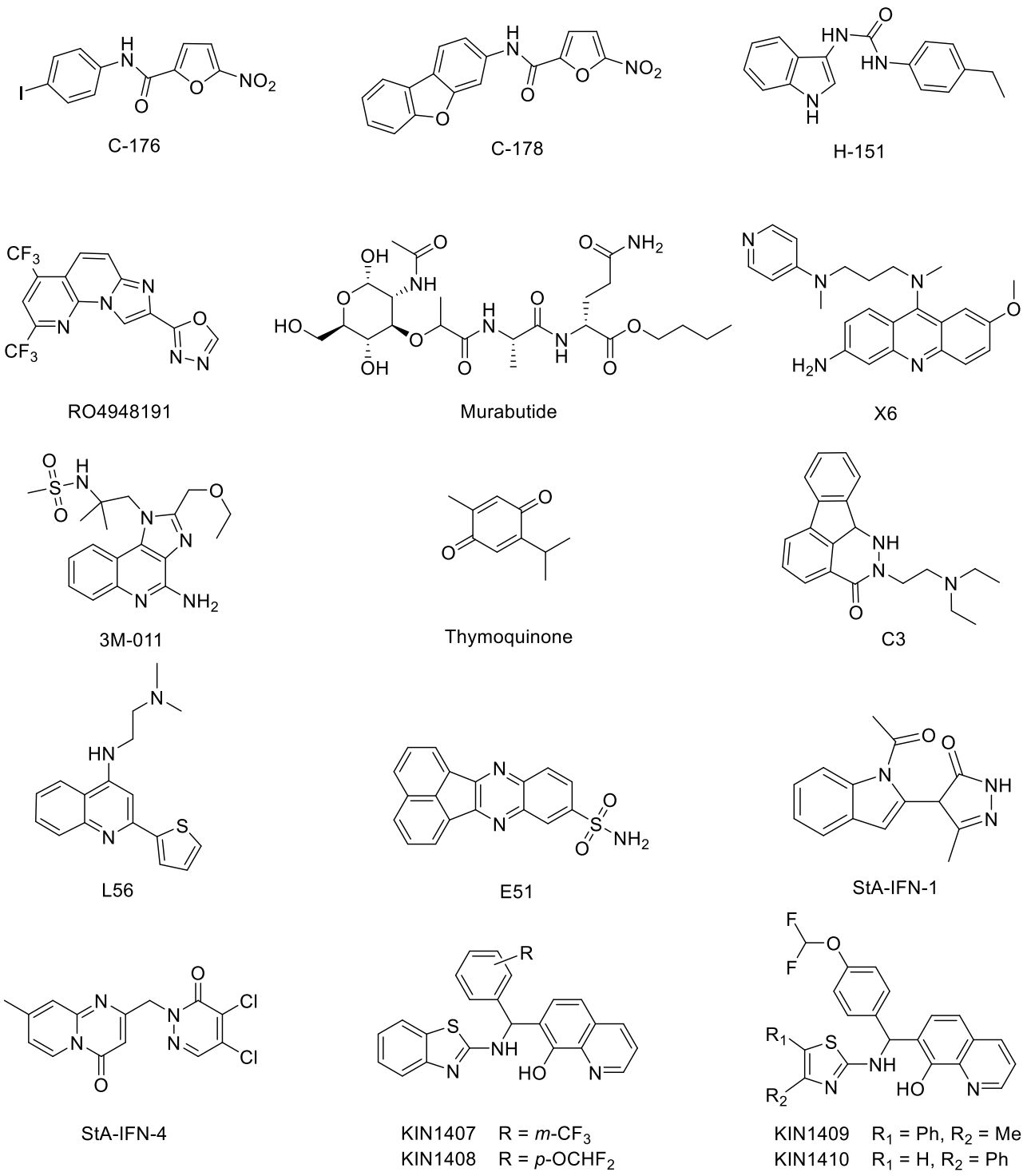


Figure 3. Natural and synthetic compounds that modulate the biological activity of Type I Interferons.

Conclusions

The synthesis of ten structurally different types of compounds derived from nitropolyhaloalkenes, that modulate the biological activity of Type I Interferons (IFN-I) is described starting from trichloroethene – a versatile solvent.

Three of them (bornylamine derivative **P6C11**, chlorothiophene **P28G6**, and nitroindole **P28F7**) are valuable starting points to design even more potent derivatives in order to obtain highly active substances and modulate the dual clinic effect of these cytokines. The described inhibitory activity of IFN-I, potentiate the use of these compounds as candidates to counteract the negative inflammatory response triggers by human IFN in some autoimmune diseases like systemic lupus erythematosus, amyotrophic lateral sclerosis, among others, where the production of this cytokine is exacerbated and is considered to be one of the causes of their etiopathologies.

Experimental Section

General information. Solvents and reagents were used as received from commercial sources without further purification. TLC was performed with Merck aluminum-backed TLC plates with silica gel 60, F254. Flash column chromatography was performed with Macherey–Nagel silica gel 60 M (0.040–0.063 mm) with appropriate mixtures of petroleum ether (PE, boiling range 60–70 °C) and ethyl acetate as eluents. Melting points (m.p.) were determined in capillary tubes with a Büchi B-520 instrument and were not corrected. FTIR spectra were recorded with a Bruker "Alpha-T" spectrometer with solid compounds measured as KBr pellets. ATR-IR spectra were measured on the same instrument with a Bruker "Alpha Platinum ATR" single reflection diamond ATR module. ^{1}H NMR and ^{13}C NMR spectra at 400 and 100 MHz, respectively, were recorded with an "Avance" 400 MHz FT–NMR spectrometer (Bruker, Rheinstetten, Germany). ^{1}H and ^{13}C NMR spectra were referenced to the residual solvent peak: $CDCl_3$, δ = 7.26 (^{1}H) and 77.0 ppm (^{13}C); DMSO-d_6, δ = 2.50 (^{1}H), and 39.7 ppm (^{13}C). Mass spectra were obtained with a Hewlett–Packard "MS 5989B" spectrometer, usually in direct mode with electron impact (70 eV). For chlorinated and brominated compounds, all peak values of molecular ions and fragments refer to the isotope ^{35}Cl and ^{79}Br. High resolution mass spectra were recorded with a Waters mass spectrometer "VG Autospec" (EI), with a WATERS mass spectrometer "Q-Tof Premier" coupled with a Waters "Acquity UPLC" (ESI), or with a Micromass mass spectrometer "LCT" coupled with a Waters "Alliance 2965 HPLC" (ESI) at the Institute of Organic Chemistry, Leibniz University of Hannover.

***(1Z,E,3E)*-*N*,*N*-Dibenzyl-4-bromo-3,4-dichloro-2-nitro-1-(phenylthio)buta-1,3-dien-1-amine (P5D7)**. Compounds **2**-**7** were prepared from trichloroethene **1** as previously reported,[10-12] and their spectroscopic characterizations agreed fully with the published data. To a suspension of sulfane **7** (195 mg, 0.50 mmol) in 10 mL MeOH at -10 °C a solution of dibenzylamine (198 mg, 1.00 mmol) in 4 mL MeOH was added dropwise within 10 min. The resulting mixture was stirred for 1 h at the same temperature, then kept at rt for 10 h. Subsequently, the supernatant liquid was concentrated *in vacuo* to a volume of 5 mL. After cooling to 0 °C the precipitate was filtered off, washed with H_2O(2 × 10 mL) and cold MeOH (2 mL) and finally dried under reduced pressure to yield 212 mg (77%) of the desired product **P5D7**, as a yellow solid, as mixture of 1-*Z*- and 1-*E*-isomers (1: 1). The isomers could not be separated; m.p. 163 – 165 °C. IR (KBr): ν = 3035, 2933, 1517 (NO_2), 1456, 1424, 1376, 1264 (NO_2), 1177, 1080, 1023, 871, 812, 772, 749, 694, 590, 495 cm^{-1}.^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.50 – 7.27 (m, 11 H), 7.13 – 7.01 (m, 4 H), 4.92 – 3.82 (m, 4 H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.7 and 166.1 (SCN), 133.9 (CH_2-Cq), 133.1 and 133.0 (CH), 130.6 and 129.1 (CCl), 130.06 and 130.04 (SCq), 129.97 and 129.93 (CH), 129.75 and 129.72 (CH), 128.9 (CH), 128.86 and 128.83 (CH), 128.70 and 128.65 (CH), 128.51 and 128.49 (CH), 122.3 and 120.5 (CNO_2), 113.0 and 112.3 (CBr), 58.2 and 58.1 (CH_2). MS: *m/z* (%) = 548 (2) [M^+], 512 (2) [M - HCl]$^+$, 469 (4) [M - Br]$^+$, 406 (4), 359 (3) [M - Br - PhS]$^+$, 91 (100). HRMS-EI: calcd. for $C_{24}H_{19}BrCl_2N_2O_2S$ [M^+]: 547.9728; found: 547.9724.

(*E*)-2,4-Dimethyl-*N*-(1,3,4,4-tetrachloro-1-nitrobuta-1,3-dien-2-yl)aniline (P5H10). Compounds **8**–**11** were prepared from butene **2** as previously reported,[13-16] and their spectroscopic characterizations agreed fully with the published data. To a solution of nitrodiene **11** (271 mg, 1.00 mmol) in 10 mL MeOH at -30 °C a solution of 2,4-dimethylaniline (242 mg, 2.00 mmol) in 2 mL MeOH was added dropwise within 10 min. The mixture was stirred for 1 h at this temperature, then kept at rt for 2 h. Subsequently, after addition of 30 mL of cold H_2O, 1 mL conc. hydrochloric acid was added dropwise. After 5 min stirring, the mixture was extracted with $CHCl_3$ (3 × 20 mL). The combined organic layers were washed with brine and dried over anhydrous calcium chloride. The crude product was purified by means of column chromatography using a mixture of PE/EtOAc (5 : 1) as eluent. Evaporation of solvents gave 267 mg of diene **P5H10** as a yellow solid. Yield 75%; m.p. 107 – 108 ℃. IR (KBr): *v* = 3213, 2954, 2922, 1602, 1563 (NO_2), 1482, 1404, 1367 (NO_2), 1239, 1181, 1113, 1055, 947, 890, 823, 738, 678, 596, 559 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 11.30 (br s, 1H, NH), 7.15 – 6.93 (m, 3 H), 2.35 (s, 3 H, Me), 2.32 (s, 3 H, Me). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 148.2 (Cq-NH), 138.7 (Cq(Ar)-NH), 132.6 (Cq-Me), 132.4 (Cq-Me), 131.8 (CH), 127.6 (CH), 127.3 and 120.6 (C_2Cl_3), 124.1 (CH), 115.6 (CNO_2), 21.0 (Me), 17.8 (Me). MS: *m/z* (%) = 354 (26) [M^+], 319 (7) [M - Cl]$^+$, 308 (23) [M - NO_2]$^+$, 273 (76) [M - NO_2 - Cl]$^+$, 203 (74) [M - NO_2 - $C_6H_3Me_2$]$^+$, 105 (100) [$C_6H_3Me_2$]$^+$. HRMS-EI: calcd. for $C_{12}H_{10}Cl_4N_2O_2$ [M^+]: 353.9496; found: 353.9497. Interestingly, nitrobutadiene **P5H10** was used for the screening for lifespan-altering compound of eukaryotic organisms.[17]

3,4,4-Trichloro-2-nitro-*N*,*N'*-bis(1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl)buta-1,3-diene-1,1-diamine (P6C11). Compounds **12** and **13** were prepared from butene **2** as previously reported,[13,18] and their spectroscopic characterizations agreed fully with the published data. Bornylamine derivative **P6C11** was obtained from 0.50 mmol nitrodiene **13** and 2.00 mmol bornylamine in Et_2O at -50 °C as previously reported.[19] Light brown solid. Yield 182 mg, 72%; m.p. 141 – 142 ℃. IR (KBr): *v* = 3322, 2956, 2881, 1605, 1565, 1561 (NO_2), 1455, 1390, 1338 (NO_2), 1263, 1192, 1138, 1080, 956, 864, 797, 695, 571, 531 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 10.80 (br s, 2H, NH), 3.77 – 3.35 (m, 2 H, CH-NH), 1.96 – 1.68 (m, 8 H, CH, CH_2), 1.35 – 1.05 (m, 6 H, CH, CH_2), 1.00 (s, 3 H, Me), 0.95 (s, 6 H, Me), 0.93 (s, 3 H, Me), 0.88 (s, 6 H, Me). MS: *m/z* (%) = 503 (1) [M^+], 487 (1) [M - O]$^+$, 468 (2) [M - Cl]$^+$, 432 (3) [M - 2HCl]$^+$, 152 (80) [$C_{10}H_{17}NH$]$^+$, 137 (100) [$C_{10}H_{17}$]$^+$. $C_{24}H_{36}Cl_3N_3O_2$ (504.92): calcd. C 57.09, H 7.19, N 8.32, Cl 21.06; found C 56.87, H 7.24, N 8.31, Cl 21.35.

2-(1,1-Dichloro-3-(1,3-dithiolan-2-ylidene)-3-nitroprop-1-en-2-yl)-1,2,3,4-tetrahydroisoquinoline (P28H3). Dithiolane **14** was prepared from nitrodiene **13** as previously reported,[20] and its spectroscopic characterizations agreed fully with the published data. To a stirred suspension of 293 mg (1.00 mmol) of **14** in 15 mL of EtOH 400 mg (3.00 mmol) 1,2,3,4-tetrahydroisoquinoline was added at 10 °C. The resulting mixture was refluxed for 32 h. Subsequently, after addition of 70 mL of cold H_2O, 1 mL conc. hydrochloric acid was added dropwise. After stirring for 10 min, the precipitate was filtered off, washed with H_2O (2 × 10 mL) and cold MeOH (1 mL) and finally dried under reduced pressure to yield 331 mg (85%) of the tetrahydroisoquinoline **P28H3** as a yellow solid; m.p. 121 – 123 °C. IR (KBr): *v* = 2932, 2843, 2803, 1581, 1525 (NO_2), 1460, 1383, 1267 (NO_2), 1224, 1130, 1103, 1045, 955, 855, 781, 735, 712, 562 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.19 – 7.03 (m, 3 H), 7.02 – 6.98 (m, 1 H), 4.13 (br s, 2 H, NCH_2), 3.64 – 3.47 (m, 4 H, SCH_2), 3.40 (br s, 2 H, NCH_2), 2.92 (br s, 2 H, CCH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.2 (SCS), 139.2 (NCq), 134.5 (Cq), 133.6 (Cq), 130.9 (CNO_2), 128.8 (CH), 126.2 (CH), 126.1 (CH), 125.7 (CH), 112.5 (CCl_2), 50.1 (NCH_2), 48.6 (NCH_2), 40.0 (SCH_2), 37.7 (SCH_2), 29.6 (NCH_2). MS (EI, 70 eV): *m/z* (%) = 388 (10) [M^+], 371 (42) [M - OH]$^+$, 352 (6) [M - HCl]$^+$, 210 (96) [M - NO_2 - tetrahydroisoquinoline]$^+$, 132 (71) [tetrahydroisoquinoline]$^+$, 117 (84), 104 (100). HRMS-EI: calcd. for $C_{15}H_{14}Cl_2N_2O_2S_2$ [M^+]: 387.9874; found: 387.9875.

(4-(2-Chloro-4-nitro-5-(vinylthio)thien-3-yl)piperazin-1-yl)(tetrahydrofuran-2-yl)methanone (P28G6) was synthetized in 2 steps from dithiolane **14**.

(4-(1,1-Dichloro-3-(1,3-dithiolan-2-ylidene)-3-nitroprop-1-en-2-yl)piperazin-1-yl) (tetrahydrofuran-2-yl)methanone (15). To a stirred suspension of 293 mg (1.00 mmol) **14** in 20 mL EtOH 553 mg (3.00 mmol) piperazin-1-yl(tetrahydrofuran-2-yl)methanone was added. The resulting mixture was stirred at reflux for 40 h. Subsequently, after addition of 70 mL of cold H_2O, 1 mL conc. hydrochloric acid was added dropwise. After stirring for 10 min, the precipitate was filtered off, washed with H_2O (2 × 10 mL) and dried under reduced pressure to furnish 396 mg (90%) of the piperazine **15** as a yellow solid; m.p. 91 – 93 °C. IR (ATR): *v* = 3472, 2854, 1648 (CO), 1525 (NO_2), 1448, 1344, 1275 (NO_2), 1229, 1136, 1103, 1020, 978, 913, 711, 624 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 4.72 – 4.41 (m, 1 H, OCH), 4.05 – 3.77 (m, 2 H, OCH_2), 3.76 – 3.43 (m, 8 H, 2 SCH_2, 2 NCH_2), 3.19 – 2.90 (m, 4 H, NCH_2), 2.32 – 2.15 (m, 1 H, CH_2), 2.09 – 1.75 (m, 3 H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.8 and 169.9 (C=O and SCS), 138.8 (NCq), 130.4 (CNO_2), 114.3 (CCl_2), 75.8 (CH), 69.0 (OCH_2), 49.7, 48.9, 45.6 and 42.1 (4 NCH_2), 40.1 (SCH_2), 37.6 (SCH_2), 28.3 (CH_2), 25.6 (CH_2). MS (EI, 70 eV): *m/z* (%) = 439 (22) $[M^+]$, 403 (10) $[M - HCl]^+$, 376 (20), 311 (38), 210 (100) $[M - NO_2 - N(CH_2)_4N\text{-}CO\text{-}C_4H_7O]^+$, 183 (5) $[N(CH_2)_4N\text{-}CO\text{-}C_4H_7O]^+$. HRMS-EI: calcd. for $C_{15}H_{19}Cl_2N_3O_4S_2$ $[M^+]$: 439.0194; found: 439.0190.

Over a period of 10 min at 0 °C 200 mg of an aqueous solution of NaOH (30%, 1.5 mmol) was added to 220 mg (0.50 mmol) piperazine **15** dissolved in 5 mL DMSO. After 1 h at 0 °C the mixture was stirred for additional 3 h at 10 °C. Then cold H_2O (20 mL) was added at 0 to 5 °C. The mixture was acidified to pH 1 upon dropwise addition of 5% hydrochloric acid. After extraction with $CHCl_3$ (3 × 20 mL) the combined organic layers were washed with brine and dried over anhydrous calcium chloride. The crude product was purified by means of column chromatography applying a mixture of PE/EtOAc (5 : 1) as eluent. Evaporation of solvents gave 186 mg thiophene **P28G6** as an orange solid. Yield 92%; m.p. 99 – 101 °C. IR (ATR): *v* = 2976, 2870, 1665, 1651 (CO), 1537 (NO_2), 1453, 1381, 1315 (NO_2), 1233, 1145, 1062, 1028, 988, 816, 725, 623 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 6.53 dd (1H, SC-H, *J* = 16.3, 9.1 Hz), 5.88 d (1H, =C-H_{trans}, *J* = 16.3 Hz), 5.83 d (1H, =C-H_{cis}, *J* = 9.1 Hz), 4.62 dd (1 H, OCH, *J* = 6.9, 5.6 Hz), 4.06 – 3.48 (m, 6 H, 2 NCH_2, 1 OCH_2), 3.31 – 3.02 (m, 4 H, 2 NCH_2), 2.40 – 2.18 (m, 1 H, CH_2), 2.13 – 1.80 (m, 3 H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.9 (C=O), 141.7 (SCS), 140.6 (NCq), 139.0 (CNO_2), 126.6 (CH_2), 126.5 (SCH), 119.6 (CCl), 75.8 (OCH), 69.0 (OCH_2), 50.3, 50.0, 46.2 and 42.7 (4 NCH_2), 28.4 (CH_2), 25.7 (CH_2). MS (EI, 70 eV): *m/z* (%) = 403 (100) $[M^+]$, 386 (9) $[M - OH]^+$, 368 (8) $[M - Cl]^+$, 332 (5) $[M - C_4H_6O]^+$, 322 (5) $[M - NO_2 - Cl]^+$, 288 (18). HRMS-EI: calcd. for $C_{15}H_{18}ClN_3O_4S_2$ $[M^+]$: 403.0427; found: 403.0428.

4-(3-(Dichloromethyl)-4-nitro-1-(3-(trifluoromethyl)phenyl)-1*H*-pyrazol-5-yl)morpholine (P28E1). To a stirred suspension of 405 mg (1.00 mmol) of 4-(1-(benzotriazol-1-yl)-3,4,4-trichloro-2-nitrobuta-1,3-dien-1-yl)morpholine (**16**)[22] in 10 mL MeOH a solution of 388 mg (2.20 mmol) of (3-(trifluoromethyl)phenyl)hydrazine in 5 mL MeOH was added at 10 °C. The resulting mixture was stirred at 40–45 °C for 6 h. Subsequently, after addition of 80 mL of cold H_2O, 1 mL conc. hydrochloric acid was added dropwise. After 5 min stirring, the mixture was extracted with $CHCl_3$ (3 × 20 mL). The combined organic layers were washed with brine and dried over anhydrous calcium chloride. The crude product was purified by means of column chromatography applying a mixture of PE/EtOAc (10: 1) as eluent. Evaporation of solvents gave 267 mg diene **P28E1** as a yellow oil, which solidified in a refrigerator. Yield 319 mg (75%), m.p. 76 – 77 °C. IR (KBr): *v* = 3035, 2872, 1562 (NO_2), 1506, 1328 (NO_2), 1180, 1125, 1068, 937, 865, 742, 691 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.92 (s, 1H, CH), 7.89 – 7.78 (m, 2 H, CH), 7.76 – 7.65 (m, 1 H, CH), 7.59 (s, 1H, $CHCl_2$), 3.93 – 3.79 (m, 4 H, OCH_2), 3.37 – 3.24 (m, 4 H, NCH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 153.0 (NCN), 139.3, 139.0, 131.9 (q, $\underline{C}CF_3$, $^2J_{C\text{-}F}$= 33.6 Hz), 130.3 (q, CH, $^4J_{C\text{-}F}$= 1.5 Hz), 129.9 (CH), 127.1 (q, CH, $^3J_{C\text{-}F}$= 3.7 Hz), 124.3 (q, CH, $^3J_{C\text{-}F}$= 3.9 Hz), 123.1 (d, CF_3, $^1J_{C\text{-}F}$= 272.7 Hz), 122.6 (CNO_2), 66.4 (OCH_2), 57.7 ($CHCl_2$), 49.9 (NCH_2). MS (EI, 70 eV): *m/z* (%) = 424 (100) $[M^+]$, 407 (34) $[M - OH]^+$, 389 (40) $[M - Cl]^+$. HRMS-EI: calcd. for $C_{15}H_{13}Cl_2F_3N_4O_3Na$ $[M + Na]^+$: 447.0209; found: 447.0224.

(*Z, E*)-N'-((5-((4-chlorophenyl)amino)-4-nitro-1*H*-pyrazol-3-yl)methylene)acetohydrazide (P28E9). A solution of 281 mg (1.00 mmol) of *N*-(4-chlorophenyl)-3-(hydrazineylidenemethyl)-4-nitro-1*H*-pyrazol-5-amine (**18**)[23] in

3 mL acetic anhydride was stirred at rt for 0.5 h. After cooling to 0 °C the precipitate was filtered off, washed with cold MeOH (2 x 2 mL) and H_2O (2 mL) and finally dried under reduced pressure to yield 212 mg (77%) of the desired product **P28E9**, red solid, as mixture of *Z*- and *E*-isomers (4 : 10). The isomers could not be separated; m.p. 265 – 267 °C. IR (KBr): ν = 3388, 3252, 1672, 1599, 1564 (NO_2), 1492, 1371 (NO_2), 1288, 1025, 822, 664, 552 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ = 14.08 (br s, 0.4 H, NH), 13.87 (br s, 1 H, NH), 11.93 (br s, 0.4 H, NH), 11.74 (br s, 1 H, NH), 8.78 (br s, 1.4 H, NH), 8.61 (s, 0.4 H, CH=N), 8.39 (s, 1 H, CH=N), 7.77 (d, 2.8 H, *J* = 8.6 Hz, CH), 7.35 (d, 2.8 H, *J* = 8.6 Hz, CH), 2.25 (s, 3 H, CH_3), 2.01 (s, 1.2 H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 172.9 and 166.3 (C=O), 147.3 and 147.2 (Cq), 139.5 and 139.4 (Cq), 136.2 and 135.9 (Cq), 133.7 and 129.8 (CH=N), 128.7 (CH), 125.1 and 125.0 (C-Cl), 119.8 (CH), 119.4 and 119.1 (CNO_2), 21.9 and 20.3 (CH_3). MS: *m/z* (%) = 322 (100) [M^+], 280 (58) [M - Ac]$^+$, 263 (95) [M - Ac - OH]$^+$. HRMS-EI: calcd. for $C_{12}H_{11}ClN_6O_3Na$ [M + Na]$^+$: 345.0473; found: 345.0488.

Methyl 2-nitro-2-(5-nitro-1,3-dihydro-2*H*-benzoimidazol-2-ylidene)acetate (P6H1) was synthetized in 2 steps from azole **19**.

1,1-Dichloro-1,3-dinitro-3-(5-nitro-1,3-dihydro-2*H*-benzoimidazol-2-ylidene)propan-2-one (20). To 50 mL of 65 % nitric acid 5.00 g (16.31 mmol) of 2-(2,3,3-trichloro-1-nitroallylidene)-2,3-dihydro-1*H*-benzoimidazole (**19**)[24] was added at 10 °C within 10 min. The resulting mixture was stirred for 2 h at 10–15 °C and at rt overnight and then poured onto 350 g ice under stirring. The precipitate was filtered off, washed with H_2O (5 x 20 mL) and Et_2O (10 mL) and finally dried under reduced pressure to give 3.45 g (56%) of ketone **20**, yellowish solid; m.p. 199 – 201 °C. IR (KBr): ν = 3563, 3325, 1582, 1556, 1533 (NO_2), 1484, 1343 (NO_2), 1277, 1163, 1070, 945, 910, 760, 646, 541 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ = 13.89 (br s, 2 H, NH), 8.57 (dd, 1 H, *J* = 2.3, 0.5 Hz, CH), 8.31 (dd, 1 H, *J* = 9.1, 2.3 Hz, CH), 7.93 (dd, 1 H, *J* = 9.1, 0.5 Hz, CH). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 171.1 (C=O), 147.8 (Cq), 144.5 (Cq), 134.7 (Cq), 130.2 (Cq), 120.7 (CH), 114.5 (CH), 112.1 (Cl_2CNO_2), 110.6 (CNO_2), 110.0 (CH). MS: *m/z* (%) = 377 (3) [M^+], 331 (3) [M - NO_2]$^+$, 249 (10) [M - Cl_2CCNO_2]$^+$, 221 (8) [M - $C(O)CCl_2NO_2$]$^+$, 205 (22) [M - $C(O)CCl_2NO_2$ - O]$^+$, 188 (100) [M - $C(O)CCl_2NO_2$ - O - OH]$^+$. HRMS-EI: calcd. for $C_{10}H_5Cl_2N_5O_7Na$ [M + Na]$^+$: 399.9458; found: 399.9456.

To 20 mL MeOH was added at rt 378 mg (1.00 mmol) of ketone **20** and the resulting mixture was refluxed for 6 h. Subsequently, the supernatant liquid was concentrated in vacuo to a volume of about 5 mL and cooled to 10 °C. The precipitate was filtered off, washed mit H_2O (3 mL) and cold MeOH (3 mL) and finally dried under reduced pressure to yield 196 mg (70%) compound **P6H1** as yellowish solid, m.p. 227 – 229 °C. IR (KBr): ν = 3249, 1706, 1623, 1579, 1530, 1484, 1341 (NO_2), 1261, 1150, 1108, 901, 734, 608, 545 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ = 13.10 (br s, 2 H, NH), 8.40 (d, 1 H, *J* = 1.8 Hz, CH), 8.14 (dd, 1 H, *J* = 8.8, 1.8 Hz, CH), 7.72 (d, 1 H, *J* = 8.8, CH). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 164.1 (C=O), 149.8 (CNO_2), 143.7 (Cq), 135.1 (Cq), 130.3 (Cq), 120.0 (CH), 113.3 (CH), 109.0 (CH), 106.0 (CNO_2), 51.9 (OCH_3). MS: *m/z* (%) = 280 (12) [M^+], 249 (10) [M - OMe]$^+$, 203 (12) [M - OMe - NO_2]$^+$, 144 (75). HRMS-EI: calcd. for $C_{10}H_8N_4O_6Na$ [M + Na]$^+$: 303.0336; found: 303.0341.

2-((2-Chlorophenyl)amino)-3-nitropyrazolo[1,5-*d*][1,2,4]triazin-7(6*H*)-one (P28H7) was synthesized in two steps from diene **21**.

Methyl 2-((3-((2-chlorophenyl)amino)-4-nitro-1*H*-pyrazol-5-yl)methylene)hydrazine-1-carboxylate (22). To a suspension of 454 mg (1.00 mmol) of 3,4,4-trichloro-*N,N*'-bis(2-chlorophenyl)-2-nitrobuta-1,3-diene-1,1-diamine (**21**)[26] in 20 mL of MeOH was added 721 mg (8.00 mmol) of methyl hydrazinecarboxylate and the resulting mixture was refluxed for 12 h. Subsequently, the supernatant liquid was concentrated in vacuo to a volume of about 10 mL and cooled to rt. The precipitate was filtered off, washed mit MeOH (3 mL), 5% hydrogen chloride (3 x 5 mL), H_2O (5 mL) and again with MeOH (3 mL) and finally dried under reduced pressure to yield 298 mg (88%) of pyrazole **22** as single isomer, orange solid, m.p. 225 – 227 °C. IR (KBr): ν = 3361, 3268, 1716 (CO), 1600, 1531 (NO_2), 1453, 1365 (NO_2), 1247, 1169, 1048, 953, 744, 584 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6):

δ = 11.80 (br s, 1 H, NH), 10.05 (br s, 1 H, NH), 9.05 (br s, 1 H, NH), 8.51 (s, 1 H, HC=N), 8.39 (d, 1 H, *J* = 8.0 Hz, CH), 7.52 (dd, 1 H, *J* = 8.0, 1.4 Hz, CH), 7.39 (ddd, 1 H, *J* = 8.0, 7.6, 1.4 Hz, CH), 7.02 (ddd, 1 H, *J* = 7.9, 7.6, 1.4 Hz, CH), 3.74 (s, 3 H, OMe). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 153.7 (CO), 146.7 (Cq), 136.3 (Cq), 136.1 (Cq), 131.9 (HC=N), 129.4 (CH), 128.5 (CH), 122.5 (CH), 120.4 (CNO_2), 119.6 (Cq), 118.3 (CH), 52.6 (OCH_3). MS: *m/z* (%) = 338 (16) $[M^+]$, 307 (21) $[M - OMe]^+$, 279 (25) $[M - CO_2Me]^+$, 136 (100). HRMS-EI: calcd. for $C_{12}H_{11}ClN_6O_4Na$ $[M + Na]^+$: 361.0423; found: 361.0426.

To a solution of 170 mg (0.50 mmol) of pyrazole **22** in 10 mL of DMF 98 mg (1.50 mmol) of sodium azide was added at rt, and the resulting mixture was stirred at 110 °C for 8 h. Subsequently, after cooling to rt and addition of 60 mL of cold H_2O, 1 mL conc. hydrochloric acid was added dropwise. After 5 min stirring, the precipitate was filtered off, washed with 5 mL $H_2O$and cold MeOH (2 x 2 mL) and finally dried under reduced pressure to give 113 mg (74%) of the desired triazinone **P28H7** as yellow solid; m.p. 221 – 223 °C. IR (KBr): ν = 3312, 1766, 1725 (CO), 1605, 1566 (NO_2), 1476, 1372 (NO_2), 1322, 1172, 1053, 904, 764, 642 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.74 (br s, 1 H, NH), 9.19 (br s, 1 H, NH), 8.71 (s, 1 H, HC=N), 8.42 (d, 1 H, *J* = 8.0 Hz, CH), 7.57 (d, 1 H, *J* = 7.9 Hz, CH), 7.46 (dd, 1 H, *J* = 8.0, 7.7 Hz, CH), 7.12 (dd, 1 H, *J* = 7.8, 7.6 Hz, CH). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 148.2 (Cq), 142.6 (Cq), 135.3 (Cq), 133.8 (Cq), 129.6 (CH), 128.6 (CH), 128.3 (CH), 124.1 (CH), 121.8 (Cq), 119.5 (CH), 115.9 (CNO_2). MS: *m/z* (%) = 306 (28) $[M^+]$, 271 (65) $[M - Cl]^+$, 255 (6) $[M - Cl - O]^+$, 78 (100). HRMS-EI: calcd. for $C_{11}H_7ClN_6O_3Na$ $[M + Na]^+$: 329.0160; found: 329.0169.

7-Methyl-3-nitro-*N*-(*o*-tolyl)-1*H*-indolyl-2-amine (P28F7) was synthetized in 3 steps from ethene **1**. Nitroethene **23** was prepared as previously reported,[31] and its spectroscopic characterizations agreed fully with the published data.

2-Chloro-2-nitro-*N*,*N*'-di-*o*-tolylethene-1,1-diamine (24). To a solution of *o*-toluidine (4.29 g, 40.00 mmol) in 70 mL MeOH at -30 °C a solution of nitroethene **23** (1.76 g, 10.00 mmol) in 10 mL MeOH was added dropwise within 10 min. The resulting mixture was stirred for 1 h at the same temperature, then kept at rt for 3 h. Subsequently, the supernatant liquid was concentrated in vacuo to a volume of about 15 mL. After cooling to 0 °C the precipitate was filtered off, washed with H_2O (2 × 50 mL) and cold MeOH (10 mL) and finally dried under reduced pressure to yield 2.64 g (83%) of the enamine **24** as a yellow solid; m.p. 126 – 127 °C. IR (ATR): *v* = 3375, 2918, 1614, 1592, 1522 (NO_2), 1453, 1383 (NO_2), 1347, 1219, 1177, 1118, 1072, 858, 772, 748, 714, 591, 497 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.49 (br s, 2H, NH), 7.05 – 6.81 (m, 8 H), 2.17 (s, 6 H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 154.1 (NCN), 135.9 (NCq), 133.1 (MeCq), 130.4 (CH), 126.8 (CH), 126.1 (CH), 125.6 (CH), 105.5 (CNO_2), 17.8 (CH_3). MS: *m/z* (%) = 317 (25) $[M^+]$, 302 (2) $[M - Me]^+$, 287 (3) $[M - 2 Me]^+$, 271 (53) $[M - NO_2]^+$, 235 (46) $[M - NO_2 - HCl]^+$, 91 (100) [*o*-tolyl$^+$]. HRMS-EI: calcd. for $C_{16}H_{16}ClN_3O_2$ $[M^+]$: 317.0931; found: 317.0934.

The solution of enamine **24** (318 mg, 1.00 mmol) in 10 mL DMSO was heated at 70 – 75 °C for 3 h. Subsequently, after addition of 50 mL of cold H_2O, 0.5 mL conc. hydrochloric acid was added dropwise. After stirring for 10 min, the precipitate was filtered off, washed with H_2O (2 × 10 mL) and MeOH (3 mL) and finally dried under reduced pressure to yield 202 mg (72%) of the nitroindole **P28F7** as a light yellow solid; m.p. 223 – 225 °C. IR (ATR): *v* = 3114, 3001, 1632, 1598, 1512 (NO_2), 1433, 1399, 1313 (NO_2), 1275, 1208, 1154, 1085, 896, 788, 741, 729, 638, 566, 456 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.15 (br s, 1H, NH), 10.09 (br s, 1 H, NH), 7.79 (d, *J* = 8.0 Hz, 1 H), 7.54 – 7.22 (m, 4 H), 7.09 (d, *J* = 7.5 Hz, 1 H), 6.95 (d, *J* = 7.4 Hz, 1 H), 2.35 (s, 3 H, CH_3), 2.30 (s, 3 H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 148.6 (Cq), 135.7 (Cq), 133.7 (Cq), 131.2 (CH), 130.9 (Cq), 127.3 (CH), 127.2 (CH), 125.6 (CH), 125.4 (CH), 123.0 (CH), 121.1 (Cq), 121.0 (Cq), 116.4 (CH), 112.1 (CNO_2), 17.9 (CH_3), 17.1 (CH_3). MS: *m/z* (%) = 281 (100) $[M^+]$, 247 (60), 234 (38) $[M - HNO_2]^+$, 219 (24). HRMS-EI: calcd. for $C_{16}H_{15}N_3O_2$ $[M^+]$: 281.1164; found: 281.1164.

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Supplementary Material

Copies of ^{1}H NMR and ^{13}C NMR spectra are given in the Supplementary Material associated with the manuscript.

Notes

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