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Biologically Active Trifluoromethyl-Substituted Metallocene Triazoles: Characterization, Electrochemistry, Lipophilicity, and Cytotoxicity

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We report the facile synthesis of four different trifluoromethylated metallocene triazoles and their biological evaluation (M = Fe and Ru). The cytotoxicity of all compounds was evaluated using MCF-7, HT-29, PT-45 and GM5657 cells and IC_{50} values as low as 33 μM were found. It was shown that the metallocene moiety is crucial for the cytotoxic effect. The electrochemical behavior of triazoles **3**–**6** was investigated by cyclic voltammetry. These electrochemical measurements revealed that all triazoles are less prone to oxidation than ferrocene. Moreover, Log P determination by RP-HPLC showed increased lipophilicity for the fluorinated derivatives with Log P values up to 3.8. Furthermore, successful bioconjugation could be achieved by coupling triazole **7** to the amino acid L-leucine.

Introduction

During the last several decades metallocene derivatives, particularly those based on ferrocene, have attracted considerable attention as chemotherapeutic agents and antibiotics.[1] However, among these compounds, fluorinated species are nearly unknown. On the other hand, fluorinated compounds such as 5-fluorouracil or -cytosine are among the oldest commercially available chemotherapeutic agents.[2] Consequently, the prospect of combining the virtues of metallocenes and fluorinated substituents seemed to us a promising venture by which to identify new biologically active entities. Such compounds are likely to provide interesting properties such as enhanced lipophilicity, higher acidity and altered electrostatic interactions.[3] Furthermore, the strong electron-withdrawing effect of the fluorinated substituents is likely to influence the redox characteristics of the metal center. Electron-withdrawing groups (EWGs) reduce the electron density at the ferrocene moiety inducing a higher resistance to oxidation.[4]

The fluorination of ferrocene, on the other hand, poses considerable challenges; direct introduction of fluorine or trifluoromethyl groups often implies the use of strong oxidizing agents and acidic conditions.[5] In this respect a careful choice of reagents and conditions is required to create ferrocene moieties bearing trifluoromethylated substituents. One suitable reaction is the azide–alkyne cycloaddition, otherwise known as a "click" reaction. This reaction can be performed under mild, non-oxidizing conditions, is well established, reliable and high-yielding.[6] Notably, azide–alkyne cycloadditions between metallocenes and terminal alkynes have been previously described by Astruc and co-workers.[7]

Herein, we report the synthesis, characterization and biological evaluation of several novel trifluoromethylthio- and trifluoromethyl-substituted metallocene triazoles. Application of well established "click"-chemistry enables easy access to these new compounds. In subsequent reactions the synthesized compounds could be coupled to natural amino acids to yield the first bioconjugate with these new trifluoromethylated metallocene triazoles. Moreover, all new trifluoromethylated metallocene triazoles were tested for cytotoxicity on various cancer cell lines, the effects of electron-withdrawing substituents was revealed using cyclic voltammetry and lipophilicities were determined by RP-HPLC.[8]

Results

Synthesis and Characterization

As outlined in Scheme 1 all four triazoles **6**–**9** were synthesized using copper-catalyzed azide–alkyne cycloaddition (CuAAC) in degassed solvents. Application of a 1:1.3 ratio of azide:alkyne resulted in quantitative yields. Completeness of reaction was monitored by TLC. Purification was, typical for "click" reactions, easily done by column chromatography. All novel triazoles were characterized by elemental analysis, NMR, IR and GC–MS. The solid-state structure of **6**, **7** and **9** could be determined by X-ray crystallography.

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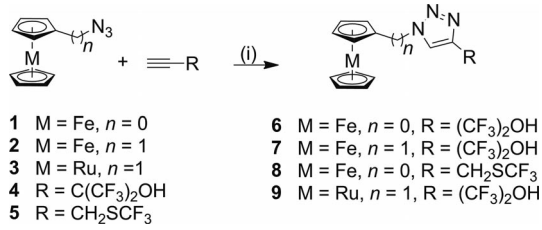


1 M = Fe, *n* = 0
2 M = Fe, *n* = 1
3 M = Ru, *n* =1
4 R = $C(CF_3)_2OH$
5 R = CH_2SCF_3

6 M = Fe, *n* = 0, R = $(CF_3)_2OH$
7 M = Fe, *n* = 1, R = $(CF_3)_2OH$
8 M = Fe, *n* = 0, R = CH_2SCF_3
9 M = Ru, *n* = 1, R = $(CF_3)_2OH$

Scheme 1. Syntheses of metallocene triazoles **6**–**9**. Conditions: (i) $CuSO_4$, sodium ascorbate, THF/H_2O (10:1), 3 d, N_2, room temp.

Azidoferrocene (**1**),[9] (azidomethyl)ferrocene (**2**),[10] (azidomethyl)ruthenocene (**3**),[11] 1,1,1-trifluoro-2-(trifluoromethyl)-3-butyn-2-ol (**4**)[12] and 3-(trifluoromethylthio)-1-propyne (**5**)[13] were chosen as starting materials for the click reactions.

Reaction of azidoferrocene (**1**) with 1,1,1-trifluoro-2-(trifluoromethyl)-3-butyn-2-ol (**4**) at room temp. gave the first trifluoromethyl-substituted ferrocene triazole **6** in 36% yield. Characterization by ^{1}H-NMR showed the aromatic protons of the substituted Cp ring as a singlet. Moreover, the OH signal at about 5.40 ppm disappeared upon addition of MeOD. In ^{19}F-NMR a singlet for the symmetric CF_3 groups was observed at –77.8 ppm. Suitable crystals for an X-ray analysis could be grown by slow evaporation of a $CHCl_3$ solution. The solid-state structure of **6** was determined by X-ray diffraction. The ORTEP plot of the molecule is shown in Figure 1. The structure was found to possess the classical "sandwich"-like appearance with an average Fe–C bond length of 2.037(6) Å for the substituted and 2.036(4) Å for the unsubstituted Cp ring. These bond lengths are fully in line with values reported in the literature for ferrocene derivatives.[14] Moreover, the Cp ring and the triazole ring are almost in plane, with a small distortion of 11.2°. Compound **6** forms dimers in the solid state through a weak hydrogen bridge between two molecules [N–(H)O distance of 2.805 Å] (Figure 2).

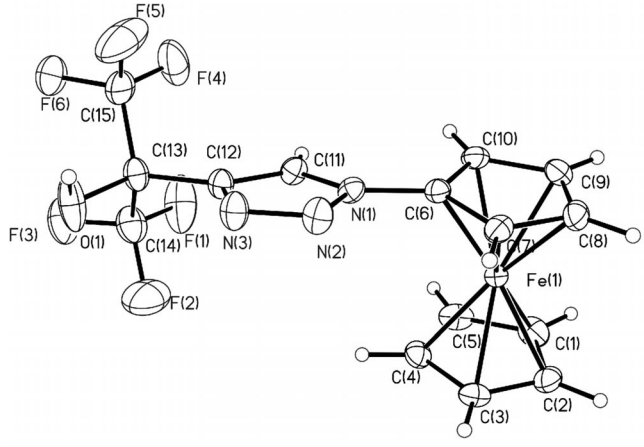


Figure 1. ORTEP plot of **6**. Selected bond lengths [Å]: Fe1–$C_{centroid}$ 1.64, Fe1–$C_{average}$ 2.04, N1–N2 1.339, N2–N3 1.309, N1–C6 1.422 C–$F_{average}$ 1.32.

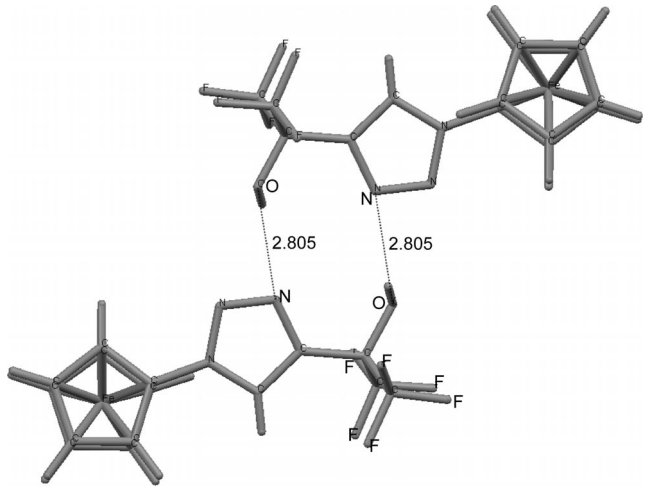


Figure 2. Intermolecular hydrogen bonding in triazole **6**; N–(H)O bond length: 2.805 Å.

The reaction of (azidomethyl)ferrocene (**2**) with 1,1,1-trifluoro-2-(trifluoromethyl)-3-butyn-2-ol (**4**) at room temp. gave triazole **7** in 95% yield. Unlike the case for compound **6**, an X-ray analysis showed that the Cp and triazole rings are not coplanar at about an angle of 53.1° due to the CH_2 unit between the triazole ring and the ferrocene moiety. Bond lengths of the ferrocene and triazole moiety are nearly the same as for **6** (Figure 3).

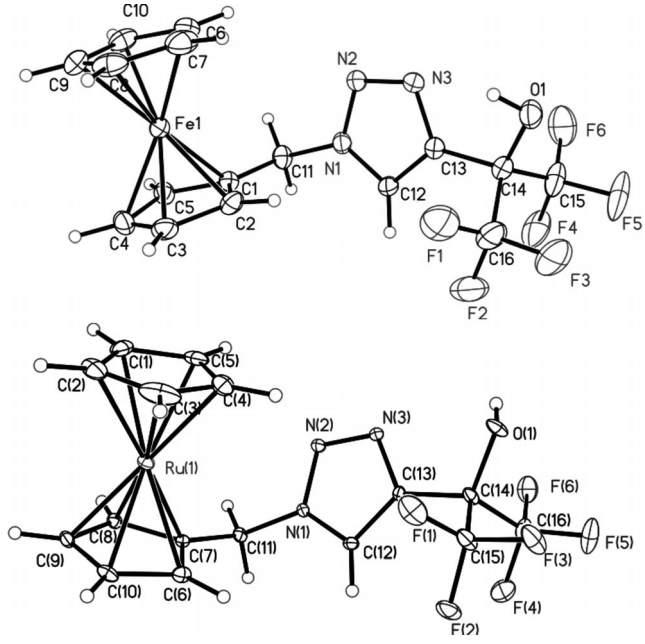


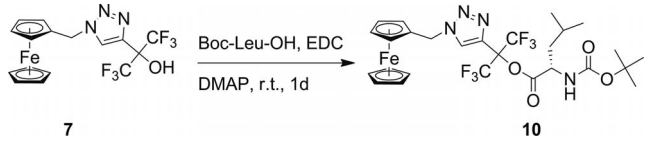
Figure 3. ORTEP plot of **7** (top) and **9** (bottom). Selected bond lengths of **7** [Å]: Fe1–$C_{centroid}$ 1.65, Fe1–$C_{average}$ 2.04, N1–N2 1.338, N2–N3 1.310, N1–C11 1.488, C–$F_{average}$ 1.33°Å. Selected bond lengths of **9** [Å]: Ru1–$C_{centroid}$ 1.81, Ru1–$C_{average}$ 2.19, N1–N2 1.344, N2–N3 1.311, N1–C1 1.481 C–$F_{average}$ 1.33°Å.

Reaction of azidoferrocene (**1**) with 3-(trifluoromethylthio)-1-propyne (**5**) at room temp. gave triazole **8** in 55% yield. In the ^{19}F-NMR a singlet for the SCF_3-group was observed at –44.5 ppm. Work-up of triazole **8** by column

chromatography however was more difficult than for **6** and **7**. The difficulty in purification of **8** may explain the relatively low yield for this triazole.

The first trifluoromethylated triazole with a ruthenocene moiety is compound **9**, which was synthesized from recently reported (azidomethyl)ruthenocene (**3**)[11] and 1,1,1-trifluoro-2-(trifluoromethyl)-3-butyn-2-ol (**4**) at room temp. (60% yield). Unlike previous observations for the ferrocene triazoles, ^{1}H NMR spectroscopy of **9** revealed the aromatic protons of the substituted Cp ring as a pair of *pseudo*-triplets. X-ray analysis revealed nearly an identical structure compared to **7**, except for the fact that the central atom is ruthenium. Ru–C bonds have an average length of 2.191(4) Å [2.179(4) Å for the unsubstituted Cp ring], which is in accordance with literature data (Figure 3).[15]

In order to test a possible bioconjugation of one of the newly synthesized triazoles we chose Boc-Leu-OH as a model system (Scheme 2). Coupling of triazole **7** with the Boc protected natural amino acid L-leucine gave bioconjugate **10**. A Steglich esterification was applied for the coupling reaction.[16] Characterization by ^{19}F-NMR showed a splitting of the signal for the fluorine atoms due to the asymmetric center at the leucine α-CH (Figure 4). Progress of the reaction could thus be conveniently monitored by ^{19}F-NMR spectroscopy. After purification by column chromatography a yield of 61% was obtained. However, it appeared during later studies that **10** hydrolyzes very easily even in slightly basic conditions and therefore, no further biological studies were carried out on this compound.



Scheme 2. Coupling of **7** to Boc-Leu-OH by use of Steglich esterification.

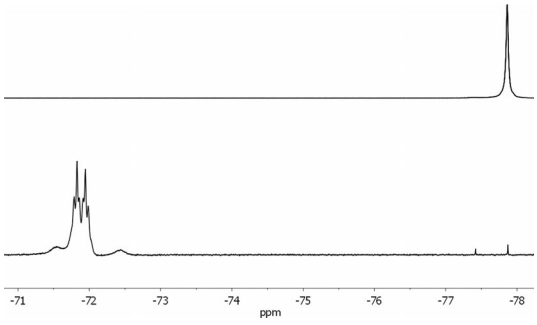


Figure 4. ^{19}F NMR of compound **10** with characteristic splitting of the CF_3 signal (lower trace) and **7** (above).

Electrochemical Studies

An electrochemical micro volume cell with a stationary glassy carbon working electrode (Ø = 3 mm), Ag/AgCl in aq. KCl (3 M) as reference electrode and a platinum wire (Ø = 2 mm) as counter electrode were used.

Cyclic voltammograms of all trifluoromethylated ferrocene triazoles revealed one-electron waves with quasi-reversible Nernst behavior (ΔE_p = 70–76 mV) and peak current ratios I_p^{Ox}/I_p^{Red} of about 1 (Table 1, Figure 5).

Table 1. Cyclic voltammetry data for compounds **6**–**8** (1 mM) in CH_3CN with $TBAPF_6$ as the supporting electrolyte (0.1 M) and ferrocene (1 mM) as standard.

	Scan rate [mV s^{-1}]	ΔE_0^f [mV] vs. $Fc^{0/+}$	ΔE_p [mV]	I_P^{Ox} [μA]	I_P^{Red} [μA]	I_P^{Ox}/I_P^{Red}
6	50	251	70	14.8	14.5	1.02
7	50	126	74	17.9	17.4	1.03
8	50	217	76	3.76	3.24	1.16

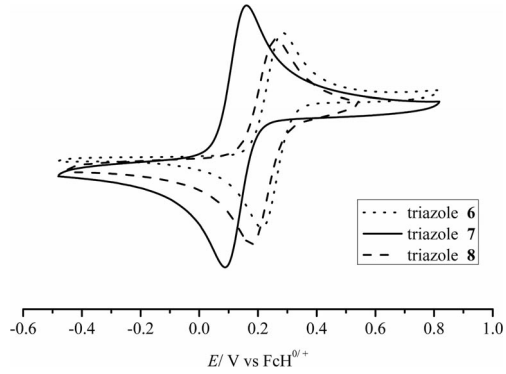


Figure 5. Cyclic voltammogram of **6**–**8** (1 mM) in CH_3CN with $TBAPF_6$ as supporting electrolyte (0.1 M) with a scan rate of 250 mV/s (**6**, **7**) and 1000 mV/s (**8**).

Triazole **6** has a half-wave potential at about 250 mV, which is in line with literature data for other ferrocene triazoles with electron-withdrawing groups (190–270 mV).[17]

Triazole **7** is more easily oxidized than **6**, as reflected by the lower half-wave potential of 126 mV, which is 112 mV higher than in literature reports for similar ferrocenyl methyltriazoles.[17a] This could be due to the $C(CF_3)_2OH$ group of the triazole ring. Nevertheless, the methylene group between the ferrocene moiety and the triazole ring shields the metal center from the EWG compared to triazole **6**.

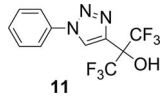
For triazole **8** a half-wave potential of 217 mV was observed, which is 23 mV lower than that observed for **6**. The difference can clearly be attributed to the varied fluorinated substituents of the triazole rings in **6** and **8** (Table 1).

Triazole **9** showed non-reversible behavior at all five scan rates. Therefore, only the peak potential E_p^{Ox} (≈ 456 mV) and the peak current I_P^{Ox} (6.2–20.7 μA) was determined. It is known that ruthenocene possesses a non-reversible redox system with a two-electron transfer in acetonitrile.[8b]

Cytotoxicity

The cytotoxicities of trifluoromethyl-substituted ferrocene triazoles **6**–**9** were evaluated using MCF-7 (breast cancer), HT-29 (colon cancer), and PT-45 (pancreas cancer)

cells. As a comparison, cytotoxicity was also checked against a non-cancerous fibroblast cell line (GM5657). To assess the importance of the metallocene moiety for the cytotoxic effect, we synthesized the metal-free compound **11** (Scheme 3). All cancer cell lines were found to be moderately sensitive to the novel metallocene triazoles ($IC_{50} \geq 33$ μM), whereas normal cells (GM5657) were unaffected at concentrations of the triazoles of up to 100 μM. Thus, some selectivity for cancer cells over normal cells seems to exist for the ferrocene triazoles investigated herein. The IC_{50} values of **11** indicate that the metallocene must have some crucial function in the cytoxicity on cancer cells (Table 2).



11

Scheme 3. A metal-free compound for comparison of the cytotoxicity data.

Table 2. IC_{50} values (in μM) of triazoles **6**–**11**.

	MCF-7	HT-29	PT-45	GM5657
6	58 ± 7.8	[a]	[a]	[a]
7	38 ± 0.5	67 ± 0.1	not tested	[a]
8	49 ± 14	55 ± 5	not tested	[a]
9	33 ± 5	41 ± 15	55 ± 25	[a]
11	[a]	[a]	[a]	[a]

[a] Inactive up to 100 μM.

Lipophilicity

Lipophilicty is one of four key properties of potential drugs according to Lipinski's "rule of five".[18] Although the ferrocene moiety already is a rather lipophilic group, incorporation of trifluoromethyl groups in the metallocene scaffold should shift the Log P to even higher values; enhanced lipophilicity would likely improve cell membrane permeability. To test these expectations, the water/octanol partition coefficients (Log P values) of **6**, **7** and **9** were determined by the reverse-phase HPLC (RP-HPLC) by the method of Minick and co-workers.[19]

The results, as shown in Table 3, indicate an increase in lipophilicity for all tested triazoles. Log P values were found to increase by about 0.4 units compared to ferrocene for all compounds. Comparison of **6** and the similar structures of **7** and **9** reveals a slight increase of lipophilicity due to the methylene group.

Table 3. Octanol/water partition coefficients of ferrocene and the triazoles **6**, **7**, and **9**.

	Log K_W	Log P	R^2
Ferrocene	2.95	3.40	0.998
6	3.31	3.75	0.999
7	3.43	3.86	0.999
9	3.38	3.81	0.996

Conclusions

In summary, the facile, high-yielding, and reliable preparation of new metallocene-containing trifluoromethylated triazoles was accomplished using "click" chemistry. We reported the synthesis, characterization and biological evaluation of four trifluoromethylated metallocene triazoles. The electrochemical behavior of triazoles **6**–**9** was investigated by cyclic voltammetry and revealed these agents to possess a greater resistance to oxidation than their non-fluorinated counterparts. Determination of Log P by PR-HPLC revealed higher lipophilicity for all novel compounds relative to ferrocene. Cytotoxicities of the new compounds were evaluated using MCF-7, HT-29, PT-45 and GM5657 cells and $IC_{50} \geq 33$ μM were found for all compounds. These assays revealed that the metallocene moiety is crucial for cytotoxicity. Because all novel trifluoromethylated metallocene triazoles show similar cytotoxicity, electrochemical behavior, and lipophilicity, a structure–activity relationship can be established. The cytotoxic activity displayed and lack of cytotoxicity against fibroblasts by these novel compounds is perhaps rationalized by formation of reactive oxygen species (ROS) inside the cell; cancer cells appear more prone to the effects of these new compounds, consistent with an ROS hypothesis. Furthermore, successful bioconjugation was demonstrated by the effective coupling of triazole **7** to L-leucine.

In future projects the introduction of fluorinated substituents will be extended to other metallocenes. Moreover, further research will focus on the bioconjugation of these novel fluorinated metallocene derivatives to larger biomolecules.

Experimental Section

General Remarks: Unless noted otherwise, all preparations were carried out under an inert atmosphere of argon or N_2 using standard Schlenk techniques. All reagents and anhydrous solvents were purchased from commercial sources and used as received. The reagents azidoferrocene (**1**),[9] (azidomethyl)ferrocene (**2**),[10] (azidomethyl)ruthenocene (**3**),[11] 1,1,1-trifluoro-2-(trifluoromethyl)-3-butyn-2-ol (**4**),[12] 3-(trifluoromethylthio)-1-propyne (**5**)[13] were prepared by literature procedures. NMR spectra were recorded at ambient temperature with Bruker DPX 200, DPX 250, DRX 400 and DRX 600 spectrometers. The chemical shifts (δ) are reported in parts-per-million (ppm) relative to the residual proton chemical shifts of the deuterated solvent set relative to external TMS. Coupling constants (J) are quoted in Hertz. IR spectra were recorded with a Bruker Tensor 27 spectrometer with an ATR unit as solid samples, wavelengths of absorption are given in cm^{-1}. Electrospray ionization mass spectra (ESI-MS) were recorded with a Bruker Esquire 6000 spectrometer. GC–MS spectra were recorded using a Shimadzu GC–MS-QP2010. Elemental analyses of ruthenium containing compounds were carried out at the laboratory for microanalytics and thermal analyses, University of Essen (Inorganic Chemistry Department), all others were carried out at the RUBiospek Biospectroscopy Department, Ruhr-Universität Bochum.

X-ray Structure Determination of 6, 7 and 9: Single crystals were obtained by slow evaporation of a $CHCl_3$ solution. The crystals (**6**, **7**: orange crystals, **9**: white needles) were placed on a glass capillary in perfluorinated oil and measured in a cold gas flow. The intensity

Table 4. Selected crystallographic data for triazoles **6**, **7**, and **9**.

	6	**7**	**9**
Empirical formula	$C_{15}H_{11}F_6FeN_3O$	$C_{16}H_{13}F_6FeN_3O$	$C_{16}H_{13}F_6RuN_3O$
Formula weight	419.02 g/mol	433.14 g/mol	478.36 g/mol
Temperature	298 K	173(2) K	173 K
Crystal system, space group	monoclinic, $P2_1/c$	triclinic, $P\bar{1}$	monoclinic, $P2_1/c$
Unit cell dimensions	a = 10.350(8) Å, α = 90°	a = 11.209(11) Å, α = 71.42(3)°	a = 16.49(2) Å, α = 90°
	b = 20.934(15) Å,	b = 13.153(11), Å	b = 11.338(16) Å,
	β = 97.140(6)°	β = 82.47(4)°	β = 90.684(16)°
	c = 7.614(6) Å, γ = 90°	c = 13.412(12) Å, γ = 67.54(2)°	c = 11.310(17) Å, γ = 90°
Volume	1637(2) Å^3	1732(3) Å^3	2114(5) Å^3
Z	4	4	4
Theta range of data collection	2.78 to 24.99°	1.97 to 25.00°	2.47 to 25.00°
Reflections collected/unique	13826/2869 (R_{int} = 0.0698)	14513/5991 (R_{int} = 0.0591)	17408/3658 (R_{int} = 0.0906)
Completeness of θ = 24.99	99.7%	98.1%	98.3%
Data/restraints/parameters	2869/0/235	5991/0/495	3658/0/248
Goodness of fit on F^2	1.051	1.083	1.055
Final R indices ($I > 2\sigma_I$)	$R1$ = 0.0533, $wR2$ = 0.1301	$R1$ = 0.0836, $wR2$ = 0.2272	$R1$ = 0.0454, $wR2$ = 0.1168
R indices (all data)	$R1$ = 0.0686, $wR2$ = 0.1402	$R1$ = 0.1051, $wR2$ = 0.2423	$R1$ = 0.0527, $wR2$ = 0.1230
Largest diff. peak and hole	0.559 and –0.374 e Å^{-3}	1.642 and –0.415e Å^{-3}	1.309 and –0.613e. Å^{-3}

data were measured with a Rigaku Mercury 375 R/M CCD (XtaLAB mini) diffractometer. The structures were solved by direct methods (SHELXS 97[20]) and refined against F^2 with all measured reflections (SHELXL97[20] and Platon/Squeeze[21]). Table 4 contains relevant crystallographic data for all crystal structures.

CCDC-878370 (for **9**), -878371 (for **6**) and -878372 (for **7**) contain the supplementary crystallographic data 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.

1,1,1-Trifluoro-2-(trifluoromethyl)-3-butyn-2-ol (4): Alkyne **4** was prepared by literature procedures, but with a modified workup method.[12,22]

An oven-dried Carius tube was purged with nitrogen while cooling, charged with a stirring bar and ethynylmagnesium bromide (20 mL, 0.5 M in THF) was added. After this, gaseous hexafluoroacetone (1 equiv.) was added to the heterogeneous mixture and was stirred vigorously for 2 d at room temp. The crude mixture was poured onto ice and concd. aqueous HCl (2 mL) was added subsequently. After distillation (boiling range 64–66 °C) the THF/alcohol azeotrope was obtained. The free alcohol was obtained from the unavoidable THF/alcohol azeotrope by addition of the azeotrope to concentrated sulfuric acid at 100 °C. Volatile material was then evaporated into a dry ice trap. Alkyne **4** was obtained as a colorless liquid. ^{1}H NMR ($CDCl_3$): δ = 3.50 (s, 1 H, OH), 2.80 (s, 1 H, CH) ppm. ^{19}F NMR ($CDCl_3$): δ = –77 (s, 6 F) ppm.

General Synthesis of Triazoles 6–9 and 11: An oven-dried 50 mL Schlenk flask was purged with nitrogen while cooling, charged with a stirring bar and the azide (1 equiv.) in THF (20 mL). $CuSO_4$ (0.05 equiv.) and sodium ascorbate (0.3 equiv.) in H_2O (2 mL) were added consecutively. The solution was degassed for 10 min. After this, the corresponding alkyne (1.3 equiv.) was added to the heterogeneous mixture and was stirred vigorously for 3 d at room temp. in the dark. TLC analysis showed the complete consumption of the azide. Dichloromethane was added to the reaction mixture, and it was washed three times with water, dried with $MgSO_4$ and the solvents evaporated in vacuo. The crude product was purified by column chromatography (silica Merck 60) using a mixture of *n*-hexane/ethyl acetate.

2-(1-Ferrocenyl-1*H*-1,2,3-triazol-4-yl)-1,1,1,3,3,3-hexafluoropropan-2-ol (6): Column chromatography: *n*-hexane/ethyl acetate (4:1). Orange solid (0.13 g, 0.31 mmol, 36%); R_f = 0.53 (*n*-hexane/ethyl acetate, 4:1). $C_{15}H_{11}F_6FeN_3O$ (419.11): calcd. C 42.99, H 2.65, N 10.03; found C 43.86, H 2.47, N 9.8. ^{1}H NMR ($CDCl_3$): δ = 7.91 (s, 1 H, $CH_{triazole}$), 5.40 (s, 1 H, OH), 4.88 (s, 2 H, CpH_2), 4.34 (s, 2 H, CpH_2), 4.24 (s, 5 H, Cp) ppm. ^{19}F NMR ($CDCl_3$): δ = –77.8 (s, 6 F) ppm. ^{13}C NMR ($CDCl_3$): δ = 137.2, 123.2, 122.4, 120.5, 117.6, 110.0, 93.0, 70.4, 67.3, 62.7, 29.7 ppm. IR (solid): $\tilde{\nu}$ = 157 ν(C–H); 1777–1629 ν(C–$C_{aromatic}$); 1400–1322 ν(C–$C_{aromatic}$); 1150 ν(C–F) cm^{-1}. ESI-MS (pos.): *m*/*z* = 419.08 ([M]$^+$, exact mass of $C_{15}H_{11}F_6FeN_3O$ = 419.02), 441.86 ([M + Na]$^+$). MS (EI): *m*/*z* = 419 [M]$^+$, 252 [M – $(CF_3)_2COH$]$^+$, 185 [Fc – H]$^+$, 121 [CpFe]$^+$, 56 [Fe]$^+$.

2-(1-Ferrocenylmethyl-1*H*-1,2,3-triazol-4-yl)-1,1,1,3,3,3-hexafluoropropan-2-ol (7): Column chromatography: *n*-hexane/ethyl acetate (4:1). Orange solid (0.33 g, 0.076 mmol, 95%); R_f = 0.4 (*n*-hexane/ethyl acetate, 4:1). $C_{16}H_{13}F_6FeN_3O$ (433.13): calcd. C 44.37, H 3.03, N 9.70; found C 44.98, H 3.30, N 9.4. ^{1}H NMR ($CDCl_3$): δ = 7.62 (s, 1 H, $CH_{triazole}$), 5.59 (s, 1 H, OH), 5.35 (s, 2 H, CH_2) 4.29 (s, 4 H, Cp), 4.19 (s, 5 H, Cp) ppm. ^{19}F NMR ($CDCl_3$): δ = –77.9 (s, 6 F) ppm. ^{13}C NMR ($CDCl_3$): δ = 137.0, 122.1 ($CH_{triazole}$), 79.8, 74.1 (quart, CF_3), 69.4 (unsubstituted Cp-Ring), 69.0, 51.9 (CH_2) ppm. IR (solid): $\tilde{\nu}$ = 3134 ν(C–H); 2925 ν(C–$C_{aromatic}$); 1538–1248 ν(C–$C_{aromatic}$); 1175 ν(C–F), 1105 ν(C–F) cm^{-1}. MS (EI): *m*/*z* = 433 [M]$^+$, 199 [$FcCH_2$]$^+$, 121 [CpFe]$^+$, 56 [Fe]$^+$.

1-Ferrocenyl-4-[(trifluoromethylthio)methyl]-1*H*-1,2,3-triazole (8): Column chromatography: *n*-hexane/ethyl acetate (2:1) Orange solid (0.02 g, 0.05 mmol, 55%); R_f = 0.4 (*n*-hexane/ethyl acetate, 2:1). $C_{14}H_{12}F_3FeN_3S$ (367.17): calcd. C 45.80, H 3.29, N 11.44, S 8.73; found C 49.51, H 3.47, N 9.46, S 5.36. ^{1}H NMR ($CDCl_3$): δ = 7.74 (s, 1 H, $CH_{triazole}$), 4.84 (s, 2 H, CH_2), 4.27 (s, 4 H, Cp), 4.22 (s, 5 H, Cp) ppm. ^{19}F NMR ($CDCl_3$): δ = –41.5 (s, 3 F) ppm. ^{13}C NMR ($CDCl_3$): δ = 141.9, 131.2, 121.0, 92.5, 69.2, 65.8, 61.2, 28.7, 23.7 ppm. IR (solid): $\tilde{\nu}$ = 2956, 2922, 2853, 2744 ν(C–H); 1583–1458 ν(C–$C_{aromatic}$); 1106 ν(C–F) cm^{-1}. MS (EI): *m*/*z* = 367 [M]$^+$, 338, 238, 199 [$FcCH_2$]$^+$, 185 [Fc – H]$^+$, 121 [CpFe]$^+$, 56 [Fe]$^+$.

1,1,1,3,3,3-Hexafluoro-2-(1-ruthenocenylmethyl-1*H*-1,2,3-triazol-4-yl)propan-2-ol (9): Column chromatography: *n*-hexane/ethyl acetate (4:1). Colorless solid (0.06 g, 0.13 mmol, 60%); R_f = 0.5 (*n*-hexane/ethyl acetate, 4:1). $C_{16}H_{13}F_6N_3ORu$ + 0.5, C_6H_{14}: calcd. C 43.76, H 3.87, N 8.06; found C 43.48, H 3.55, N 8.03. ^{1}H NMR ($CDCl_3$): δ = 7.77 (s, 1 H, $CH_{triazole}$), 5.55 (s, 1 H, OH), 5.20 (s, 2 H, CH_2) 4.66 (t, 2 H, Cp), 4.59 (t, 2 H, Cp), 4.51 (s, 5 H, Cp) ppm. ^{19}F NMR ($CDCl_3$): δ = –77.8 (s, 6 F) ppm. ^{13}C NMR ($CDCl_3$): δ =

137.1, 122.4 ($CH_{triazole}$), 122.1, 84.2, 74.1 (CF_3), 71.4 (unsubstituted Cp-Ring), 71.3 (substituted Cp-Ring), 71.1 (substituted Cp-Ring), 70.9 (substituted Cp-Ring), 70.9, 50.7 (CH_2) ppm. IR (solid): $\tilde{\nu}$ = 2922 ν(C–H); 1735 ν(C–$C_{aromatic}$); 1376–1206, 1100 ν(C–F) cm^{-1}. MS (EI): *m*/*z* = 479 $[M]^+$, 245$[Cp_2RuCH_2]^+$, 185 $[Fc – H]^+$, 167 $[CpRu]^+$.

Compound 10: An oven-dried 50 mL flask was charged with a stir bar and triazole **7** (0.022 g, 0.051 mmol) in dichloromethane (20 mL). Boc-Leu-OH (0.016 g, 0.069 mmol), EDC (0.018 g, 0.094 mmol) and DMAP (0.0087 g, 0.094 mmol) were added consecutively to the heterogeneous mixture and stirred vigorously for 1 d at room temp. The reaction mixture was washed three times with water, dried with $MgSO_4$ and the solvents evaporated in vacuo. The crude product was purified by column chromatography (silica Merck 60) using a mixture of *n*-hexane/ethyl acetate (4:1). Complex **10** was obtained as an orange oil (0.02 g, 0.031 mmol, 61%). ^1H NMR ($CDCl_3$): δ = 7.72 (s, 1 H, $CH_{triazole}$), 5.08–4.29 (m, 9 H, CpH_9), 4.66 (t, 2 H, CpH_2), 1.70 (m, 3 H), 1.44 (s, 9 H, 3 CH_3), 0.98 (s, 3 H, CH_3), 0.96 (s, 3 H, CH_3) ppm. ^{19}F NMR ($CDCl_3$): δ = –71 (t, 3 F, CF_3), –72 (t, 3 F, CF_3) ppm. ^{13}C NMR ($CDCl_3$): δ = 137.1, 122.4, ($CH_{triazole}$) 84.2, 74.1 (CF_3), 71.4 (unsubstituted Cp-Ring), 71.3 (substituted Cp-Ring), 71.1 (substituted Cp-Ring), 70.9 (substituted Cp-Ring), 51.7 (CH_2) ppm. ESI-MS (pos. mode, 70 eV): *m*/*z* = 767.9 $[M + H]^+$, 790.9 $[M + Na]^+$. Exact mass of complex cation: 766.54.

1,1,1,3,3,3-Hexafluoro-2-(1-phenyl-1*H*-1,2,3-triazol-4-yl)propan-2-ol (11): Column chromatography: *n*-hexane/ethyl acetate (4:1). Colorless solid (0.1 g, 0.32 mmol, 77%); R_f = 0.5 (*n*-hexane/ethyl acetate, 4:1). ^1H NMR ($CDCl_3$): δ = 8.15 (s, 1 H, $CH_{triazole}$), 7.77 (d, *J* = 7.49 Hz, 2 H), 7.61–7.53 (m, 3 H) 5.35 (s, 1 H, OH) ppm. ^{19}F NMR ($CDCl_3$): δ = –77.7 (s, 6 F) ppm. ^{13}C NMR ($CDCl_3$): δ = 136.9, 135.3, 129.1, 128.9, 123.1, 120.0 ppm. IR (solid): $\tilde{\nu}$ = 3154, 2932, 2853 ν(C–H); 1597–1257, 1059 ν(C–F) cm^{-1}. MS (EI): *m*/*z* = 311 $[M]^+$, 214 $[M – (CF)_3COH]^+$.

Determination of LogP Values by RP-HPLC: The RP-HPLC method of Minick and co-workers was used for LogP determination.[19] All values were determined on a Reprosil-Par C 18-AQ, 5 μm × 250 mm × 4.6 mm column. The water layer was saturated with octanol. As eluents, 3-(N-morpholino)propanesulfonic acid buffer (MOPS, 0.02 M, pH 7.4) with *n*-decylamine (0.15% v/v) and MeOH with *n*-octanol (0.25% v/v) were used. In order to calibrate the system 4-methoxyaniline, 4-bromoaniline, naphthalene and *tert*-butylbenzene were eluted at six isocratic eluent concentrations (60% to 85% of MeOH in 5% increments). The retention times, together with the dead times obtained from uracil, were used to calculate the corresponding capacity factors *k*′ H_2O/MeOH ratio. The extrapolation to 100% H_2O (0% MeOH) gave the logk_w value for each standard. All four logk_w values of the reference compounds were plotted against the literature logP values of the reference compounds to obtain a linear function that correlates both parameters. This function was used to determine all logP values from their logk_w values, which were measured in the same way as those for the references.

Electrochemical Measurements: An electrochemical micro volume cell with a stationary glassy carbon working electrode (Ø = 3 mm), Ag/AgCl in aq. KCl (3 M) as reference electrode and a platinum wire (Ø = 2 mm) as counter electrode were used. All electrochemical measurements were undertaken with 5 mL of 0.1 mM solutions of the respective trifluoromethylated metallocene triazole in acetonitrile. CV of ferrocene was performed in the same electrolyte solution, and the electrochemical half-wave potential $E_{1/2}$ of the redox couple ferrocene/ferrocenium $FcH^{0/+}$ vs. Ag/AgCl ($E_{1/2}$) (270 mV) was set to 0 mV as the reference potential for all measurements. Ferrocene was used as an internal reference for the scan rate at 50 mV s^{-1} and used as an external reference for all other scan rates to avoid an overlap of the corresponding redox processes.

The electrochemical behavior of all four triazoles **6**–**9** was examined by cyclic voltammetry (CV) at five different scan rates (50, 100, 250, 500 and 1000 mV s^{-1}).

Cytotoxicity Assays: MCF-7, HT-29, PT-45 and GM5657 cells were cultured in DMEM medium supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine, penicillin (100 U/mL), and streptomycin (100 μg/mL) in a 5% CO_2 atmosphere. Crystal violet assay was applied to determine the absolute cell numbers. All cells were seeded in 96-well cell-culture treated microtiter plates (MTP) and grown for 24 h under standard conditions. All tested compounds were dissolved in cell culture medium with 0.5% dimethyl sulfoxide (DMSO) and applied to the cells in 1, 5, 20, 50, 100, 500 μM concentrations for 72 h. Cells were fixed with 4% glutaraldehyde in 2% water for 25 min at room temp. Membranes were permeabilized by Triton X-100 (0.1%) in PBS for 10 min. and then aqueous crystal violet solution (0.04%) was added to the cells. After 30 min of mechanical shaking, the cells were washed five times with water and the crystal violet was eluted with 70% EtOH for 3 h. Absorbance was detected at 570 nm (Tecan Sapphire 2 microplate reader). The cell mass was plotted against the concentration. IC_{50} were calculated from the sigmoidal function.

Supporting Information (see footnote on the first page of this article): GC–MS spectra of compounds **6**–**9**.

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A series of new trifluoromethylated metallocene triazoles was synthesized using "click" chemistry (copper-catalyzed azide–alkyne cycloaddition). The new triazoles display suitable lipophilic character and exert promising antiproliferative activities against a range of cancer cell lines.

Heterocyclic Sandwich Complexes

Keywords: Bioinorganic chemistry / Medicinal chemistry / Cytotoxicity / Click chemistry / Fluorinated ligands / Metallocenes / Sandwich complexes / N ligands / Cycloaddition

