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Synthesis, Electrochemistry and Antitumor Activity of 1'H, 3'H(Me)-spiro-[(aza)benzimidazoline-2', 3-(1,2-diferrocenylcyclopropenes)], 2-(1,2-Diferrocenylvinyl)benz- and Azabenzimidazoles

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Abstract

A new method of synthesis of 2-(1,2-diferrocenylvinyl)benz- and azabenzimidazoles (3a-f), (4a-f) and 1'H,3'H(Me)-spiro-[(aza)benzimidazoline-2',3-(1,2-diferrocenylcyclopropenes)] (5a-f) via reactions of diferrocenyl(methylsulfanyl)cyclopropenylium iodide (1) with aromatic *o*-diamines (2a-f) in the presence of Et_3N (80˚C - 82˚C) is described. The structures of the resultant compounds are established using IR, ^1H and ^{13}C NMR spectroscopy, mass spectrometry and elemental analysis. The structure of one compound, *cis*-2-(1,2-diferrocenylvinyl)-1-methylbenzimidazole (3b), is confirmed by X-ray diffraction analysis. The electrochemical properties of compounds 3a, 3b, 3d and 5f are investigated using cyclic square wave voltammetry. Two electrochemical processes (I-II), attributed to oxidation of the ferrocene moieties, and the values of $E^{0'}(I)$, $E^{0'}(II)$, $\Delta E^{0'}(II\text{-}I)$ and comporportionation constant K_{com} are reported. The bioactivities of seven compounds 3a, 3c-f, 5d, 5f are evaluated. Compound 5f is the most active compound with a modest cytotoxic activity against six human cancer cell lines: U-251 (glioma), PC-3 (prostate cancer), K-562 (leukemia), HCT-15 (colon cancer), MCF-7 (breast cancer) and SKLU-1 (lung cancer).

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Keywords

Diferrocenyl(methylthio)cyclopropenylium, 2-(1,2-Diferrocenylvinyl)(aza)benzimidazoles, 1'H,3'H(Me)-spiro-[(aza)benzimidazoline-2',3-(1,2-diferrocenylcyclopropenes)], Electrochemistry, Antitumor Activity

1. Introduction

Diaryl- and diferrocenylcyclopropenilium cations with dialkylamino and methylsulfanyl groups in the small cycle are successfully used in organic synthesis as diferrocenyl-substituted three-carbon building blocks [1]-[7]. Reactions of such cations with carbon and nitrogen nucleophiles, which proceed via the opening of the three-membered cycle and the formation of diferrocenylvinylcarbenes, have been described [1]-[13]. On the basis of intramolecular transformations of such carbenes, researchers have developed new methods of synthesizing five- and six-membered carbo- and heterocycles, polyene compounds with two ferrocene substituents and functional groups in the molecules [4]-[13]. The interaction of diferrocenylcyclopropenylium salts with 1,2-diamines has scarcely been studied. The first study concerning the use of 2,3-diferrocenyl-1-methylsulfaylcyclopropeny- lium iodide in reactions with aliphatic 1,2-diamines for the synthesis of 2-(1,2-diferrocenylvinyl)imidazoline and imidazolidine derivatives has recently been published [13]. Relatively stable spiro-(imidazolidine)-2',3-(1,2-diferrocenylcyclopropenes) were first isolated at the same time [13]. It is known that researchers pay much attention to the synthesis of new derivatives of mono- and polycyclic imidazoles, since the imidazole ring is an important structural element in many natural compounds, alkaloids, proteins, herbicides, vitamins, medications, etc. [14]-[21].

Ferrocene-substituted nitrogen heterocycles are of special interest in search for bioactive substances [22]-[28]. Investigations of interactions between diferrocenylcyclopropenylium cations and aromatic 1,2-diamines must apparently lead to the synthesis of new diferrocenyl-substituted polycyclic imidazole derivatives, which is of interest with regard to the search for practical applications of the novel compounds in pharmacology, electrochemistry, etc.

Here we report a novel method for the synthesis of benz- and azabenzimidazoles with 1,2-diferrocenylvinyl substituents in position 2 of the heterocyclic nucleus, as well as spiro-[(aza)benzimidazoline-2',3-(1,2-diferrocenylcyclopropenes)] via reactions of diferrocenyl(methyl sulfanyl)cyclopropenylium iodide with aromatic *o*-diamines. This method has been derived from our investigations into the chemical properties of diferrocenylcyclopropene derivatives [3]-[14].

2. Experimental

2.1. Instruments

All the solvents were dried according to the standard procedures and freshly distilled before use [29]. Column chromatography was carried out on alumina (Brockmann activity III); TLC, on silica gel. The ^{1}H and ^{13}C NMR spectra were recorded on a Unity Inova Varian spectrometer (300 and 75 MHz) for solutions in $CDCl_3$, with Me_4Si as the internal standard. The IR spectra were measured on an FT-IR spectrophotometer (Spectrum RXI Perkin Elmer instruments) using KBr pellets. The mass spectra were obtained on a Varian MAT CH-6 instrument (EI MS, 70 eV). Elementar Analysensysteme LECO CHNS-900 was used for elemental analyses. All the electrochemical measurements were performed at sample concentrations of about 5×10^{-4} M in the acetonitrile solution in the presence of 0.1 M tetra-N-butylammonium hexafluorophosphate ($TBAPF_6$) using a Biologic SP-50 potentiostat/galvanostat. A typical three-electrode array was employed: a platinum disk as the working electrode, a platinum wire as the counter electrode, and a pseudo-reference electrode of silver. All the solutions were bubbled with nitrogen prior to each measurement. The cyclic wave voltammetry experiments were initiated from the open circuit potential (E_{ocp}) in the positive direction, using the scan rates from 0.1 to 2 Vs^{-1}.The current interrupt method was used for *iR* compensation. All the potentials were reported versus the Fc/Fc^{+} couple according to the IUPAC convention [30]. The unit cell parameters and the X-ray diffraction intensities for 3b were recorded on a Gemini diffractometer (detector Atlas CCD, Cryojet N_2). The crystallographic data, the parame-

ters of the X-ray diffraction experiments, and the refinements are listed in **Table 1**. The structure of compound 3b was solved by the direct method (SHELXS-97 [31]) and refined using the full-matrix least-squares on F^2.

2.2. Material and Reagents

The following reagents were purchased from Aldrich Chemical Co: 1,2-phenylenediamine 2a, 98%; N-methyl-1, 2-phenylenediamine 2b, 97%; 3,4-diaminobenzophenone 2c, 97%; 2,3-diaminopyridine 2d, 98%; 3,4-diaminopyridine 2e, 98%; 4,5-diaminopyrimidine 2f, 95%. All the ferrocenylcyclopropenes and cyclopropenyliumcations [3] [4] were synthesized according to the procedures described in the literature.

2.3. Organic Preparations

General procedure for the preparation of 2-(1,2-diferrocenylvinyl)benz-, azabenz-, diazabenzimidazoles (3a-f), (4a-f) and spiro[imi-dazolinecyclopropenes] (5a-f). The corresponding diamine (2a-f, 7 mmol) and Et_3N (3.0 ml) were added under stirring to the suspension of diferrocenyl(methylthio)cyclopropenylium iodide 1 (6 mmol) in dry benzene (100 ml). After stirring for 2 h at 80˚C, the volatiles were removed *in vacuo*; chromatography of the residue on Al_2O_3 yielded compounds (3a-f + 4a-f) and 5a-f. The geometric isomers *cis*-3a-f and *trans*-4a-f (~2:1) were separated using TLC on Al_2O_3.

Cis-2-(1,2-Diferrocenylvinyl)benzimidazole (3a). Orange powder, yield 1.23 g (40%), (TLC, hexane-CH_2Cl_2, 3:1), m.p.: 183˚C - 184˚C. IR: υ (cm^{-1}) 440, 478, 737, 767, 815, 949, 1000, 1046, 1106, 1246, 1271, 1356, 1369, 1407, 1445, 1451, 1519, 1605, 1624, 1730, 2868, 2957, 3082, 3332. ^{1}H NMR ($CDCl_3$): 4.12 (s, 5H, C_5H_5), 4.19 (s, 5H, C_5H_5), 4.22 (m, 2H, C_5H_4), 4.24 (m, 2H, C_5H_4), 4.44 (m, 2H, C_5H_4), 4.48 (m, 2H, C_5H_4), 7.28 (m, 2H, C_6H_4), 7.52 (m, 1H, C_6H_4), 7.85 (m, 1H, C_6H_4), 7.93 (s, 1H, CH=), 11.38 (bs, 1H, NH). ^{13}C NMR ($CDCl_3$): 69.55, 69.76 ($2C_5H_5$), 68.54, 69.48, 70.49, 70.56 ($2C_5H_4$), 80.91, 81.95 ($2C_{ipsoFc}$), 110.39 (CH=), 119.23, 122.51, 122.61, 134.11 (C_6H_4), 121.15, 133.21, 143.98, 153.38 (4C). IR (KBr): MS: *m/z* 512 $[M]^+$. *Calcd*. for $C_{29}H_{24}Fe_2N_2$ (512.19): C 68.00, H 4.72, N 5.47. *Found*: C 68.13, H 4.67, N 5.39%.

Trans-2-(1,2-Diferrocenylvinyl)benzimidazole (4a). Orange powder, yield: 0.61 g (20%), (TLC, hexane-CH_2Cl_2, 3:1), m.p.: 179˚C - 180˚C. ^{1}H NMR ($CDCl_3$): 4.10 (s, 5H, C_5H_5), 4.17 (s, 5H, C_5H_5), 4.20 (m, 2H, C_5H_4), 4.25 (m, 2H, C_5H_4), 4.41 (m, 2H, C_5H_4), 4.51 (m, 2H, C_5H_4), 7.32 (m, 2H, C_6H_4), 7.54 (m, 1H, C_6H_4), 7.82 (m, 1H, C_6H_4), 8.16 (s, 1H, CH=), 11.24 (bs, 1H, NH). ^{13}C NMR ($CDCl_3$): 69.43, 69.69 ($2C_5H_5$), 68.75, 69.54, 70.32, 70.43 ($2C_5H_4$), 80.17, 80.65 ($2C_{ipsoFc}$), 112.04 (CH=), 118.82, 123.16, 123.27, 135.09 (C_6H_4), 122.15, 130.19, 145.07, 153.34 (4C). MS: *m/z* 512 $[M]^+$. *Calcd*. for $C_{29}H_{24}$ Fe_2N_2 (512.19): C 68.00, H 4.72, N 5.47. *Found*: C 67.94, H 4.81, N 5.43%.

Table 1. Selected bond lengths and angles for compound 3b.

Bond lengths [Å]		Bond angles [˚]	
C(21)-H(21)	0.9500	C(22)-C(21)-H(21)	114.3
N(1)-C(23)	1.320(4)	C(22)-C(21)-C(16)	131.3(3)
N(2)-C(23)	1.380(3)	N(2)-C(23)-N(1)	112.6(3)
N(1)-C(24)	1.383(4)	N(2)-C(23)-C(22)	122.1(2)
N(2)-C(29)	1.376(4)	N(1)-C(23)-C(22)	125.2(2)
C(21)-C(22)	1.352(4)	N(1)-C(24)-C(29)	110.4(2)
C(22)-C(23)	1.481(4)	N(1)-C(24)-C(25)	129.7(3)
C(24)-C(29)	1.402(4)	C(23)-N(1)-C(24)	105.0(2)
C(24)-C(25)	1.406(4)	C(23)-N(2)-C(30)	128.4(2)
C(21)-C(16)	1.454(4)	C(29)-N(2)-C(23)	106.8(2)
N(2)-C(30)	1.462(3)	N(2)-C(29)-C(24)	105.3(2)
C(51)-C(52)	1.351(4)	C(52)-C(51)-C(46)	130.4 (3)

1'H,3'H-Spiro[benzimidazole-2',3-(1,2-diferrocenylcyclopropene)] (5a). Red crystals, yield: 0.77 g (25%), (TLC, hexane-CH_2Cl_2, 3:2), m.p.: 197˚C - 198˚C; IR: υ (cm^{-1}) 476, 781, 822, 908, 1003, 1032, 1105, 1241, 1343, 1369, 1401, 1482, 1544, 1565, 1838, 2979, 3094, 3286, 3453. ^{1}H NMR ($CDCl_3$): 4.17 (s, 10 H, $2C_5H_5$), 4.32 (m, 4 H, C_5H_4), 4.49 (m, 4 H, C_5H_4), 4.91 (bs, 2H, NH), 7.32 (d, 2H, C_6H_4, *J* = 7.5 Hz), 7.49 (dd, 2H, C_6H_4, *J* = 7.2, 7.5 Hz); ^{13}C NMR ($CDCl_3$): 65.15 (C), 66.74, 67.98 ($2C_{ipsoFc}$), 70.24 ($2C_5H_5$), 71.05, 71.34 ($2C_5H_4$), 115.03, 122.79 (C_6H_4), 130.03, 137.05 (4C). MS: *m/z* 512 $[M]^+$. *Calcd*. for $C_{29}H_{24}Fe_2N_2$ (512.19): C 68.00, H 4.72, N 5.47. *Found*: C, 68.12; H, 4.65; N, 5.54%.

Cis-2-(1,2-Diferrocenylvinyl)-1-methylbenzimidazole (3b). Orange crystals, yield: 1.13 g (35%), (TLC, hexane-CH_2Cl_2, 3:1), m.p.: 174˚C - 175˚C. IR: υ (cm^{-1}) 443, 521, 742, 822, 899, 968, 1001, 1031, 1040, 1105, 1239, 1323, 1336, 1373, 1409, 1459, 1498, 1618, 1644, 1735, 2855, 2925, 3079, 3464. ^{1}H NMR ($CDCl_3$): 3.86 (s, 3H, CH_3), 3.87 (s, 5H, C_5H_5), 4.09 (s, 5H, C_5H_5), 4.23 (m, 2H, C_5H_4), 4.25 (m, 2H, C_5H_4), 4.37 (m, 2H, C_5H_4), 4.45 (m, 2H, C_5H_4), 6.56 (s, 1H, CH=), 7.33 (m, 2H, C_6H_4), 7.42 (m, 1H, C_6H_4), 7.87 (m, 1H, C_6H_4). ^{13}C NMR ($CDCl_3$): 31.24 (CH_3), 69.21, 69.40 ($2C_5H_5$), 68.36, 69.07, 69.71, 70.14 ($2C_5H_4$), 80.60, 83.11 ($2C_{ipsoFc}$), 109.58 (CH=), 119.93, 122.27, 122.54, 133.15 (C_6H_4), 125.71, 135.17, 142.72, 156.31 (4C); MS: *m/z* 526 $[M]^+$. *Calcd*. for $C_{30}H_{26}Fe_2N_2$ (526.23): C 68.47, H 4.98, N 5.32. *Found*: C 68.56, H 4.81, N 5.24%.

Trans-2-(1,2-Diferrocenylvinyl)-1-methylbenzimidazole (4b). Orange powder, yield: 0.57 g (18%), (TLC, hexane-CH_2Cl_2, 3:1), m.p.: 181˚C - 182˚C. ^{1}H NMR ($CDCl_3$): 3.84 (s, 3H, CH_3), 3.91 (s, 5H, C_5H_5), 4.07 (s, 5H, C_5H_5), 4.24 (m, 4H, C_5H_4), 4.35 (m, 2H, C_5H_4), 4.40 (m, 2H, C_5H_4), 6.71 (s, 1H, CH=), 7.24 (m, 2H, C_6H_4), 7.38 (m, 1H, C_6H_4), 7.79 (m, 1H, C_6H_4). ^{13}C NMR ($CDCl_3$): 31.14 (CH_3), 69.23, 69.36 ($2C_5H_5$), 68.73, 69.27, 69.63, 70.04 ($2C_5H_4$), 79.84, 80.87 ($2C_{ipsoFc}$), 110.17 (CH=), 119.51, 122.13, 122.51, 132.18 (C_6H_4), 124.56, 136.61, 142.34, 154.13 (4C). MS: *m/z* 526 $[M]^+$. *Calcd*. for $C_{30}H_{26}Fe_2N_2$ (526.23): C 68.47, H 4.98, N 5.32. *Found*: C 68.39, H 5.04, N 5.37%.

1'H,3'Me-Spiro[benzimidazole-2',3-(1,2-diferrocenylcyclopropene)] (5b). Red crystals, yield: 0.70 g (22%), (TLC, hexane-CH_2Cl_2, 3:2), m.p.: 203˚C - 204˚C. IR: υ (cm^{-1}) 481, 783, 818, 907, 1001, 1028, 1103, 1240, 1342, 1371, 1405, 1479, 1541, 1562, 1832, 2811, 2987, 3091, 3279, 3445. ^{1}H NMR ($CDCl_3$): 3.59 (s, 3H, CH_3), 4.07 (s, 5H, C_5H_5), 4.09 (s, 5H, C_5H_5), 4.26 (m, 2H, C_5H_4), 4.31 (m, 2H, C_5H_4), 4.35 (m, 2H, C_5H_4), 4.52 (m, 2H, C_5H_4), 4.87 (bs, 2H, NH), 7.28 (m, 1H, C_6H_4), 7.38 (m, 2H, C_6H_4), 7.52 (m, 1H, C_6H_4). ^{13}C NMR ($CDCl_3$): 64.84 (C), 66.83, 67.11 ($2C_{ipsoFc}$), 70.13, 70.18 ($2C_5H_5$), 70.56, 70.83, 71.18, 71.23 (2 C_5H_4), 116.14, 122.33, 122.84, 135.12 (C_6H_4), 123.29, 132.21, 134.26, 136.02 (4C). MS: *m/z* 526 $[M]^+$. *Calcd*. for $C_{30}H_{26}Fe_2N_2$ (526.23): C 68.47, H 4.98, N 5.32. *Found*: C 68.56, H 4.87, N 5.28%.

Cis-6-Benzoyl-2-(1,2-diferrocenylvinyl)benzimidazole (3c). Orange powder, yield: 1.54 g (41%), (TLC, hexane-CH_2Cl_2, 3:2), m.p.: 171˚C -172˚C. IR: υ (cm^{-1}) 468, 584, 641, 718, 791, 818, 893, 978, 1000, 1027, 1043, 1106, 1178, 1226, 1248, 1282, 1298, 1318, 1390, 1409, 1442, 1476, 1515, 1598, 1615, 1648, 1736, 2854, 2924, 3087, 3302. ^{1}H NMR ($CDCl_3$): 4.11 (s, 5H, C_5H_5), 4.21 (s, 5H, C_5H_5), 4.24 (m, 2H, C_5H_4), 4.28 (m, 2H, C_5H_4), 4.45 (m, 2H, C_5H_4), 4.50 (m, 2H, C_5H_4), 7.47 - 7.53 (m, 5H, C_6H_5), 7.85 (m, 2H, C_6H_3), 8.01 (s, 1H, C_6H_3), 8.12 (s, 1H, CH=), 11.65 (bs, 1H, NH). ^{13}C NMR ($CDCl_3$): 69.66, 69.89 ($2C_5H_5$), 68.78, 69.95, 70.42, 70.79 ($2C_5H_4$), 80.34, 81.35 ($2C_{ipsoFc}$), 110.96 (CH=), 117.28, 120.54, 124.61, 128.32 (2C), 130.11 (2C), 132.09 (C_6H_5 + C_6H_3), 125.61, 133.8, 136.51, 138.31, 138.54, 155.75, 195.73 (7C). MS: *m/z* 616 $[M]^+$. *Calcd*. for $C_{36}H_{28}Fe_2N_2O$ (616.28): C 70.16, H 4.58, N 4.54. *Found*: C 70.25, H 4.67, N 4.41%.

Trans-6-Benzoyl-2-(1,2-diferrocenylvinyl)benzimidazole (4c). Orange powder, yield: 0.70 g (19%), (TLC, hexane-CH_2Cl_2, 3:2), m.p.: 187˚C - 188˚C. ^{1}H NMR ($CDCl_3$): 4.13 (s, 5H, C_5H_5), 4.19 (s, 5H, C_5H_5), 4.22 (m, 2H, C_5H_4), 4.26 (m, 2H, C_5H_4), 4.37 (m, 2H, C_5H_4), 4.48 (m, 2H, C_5H_4), 7.56 - 7.63 (m, 5H, C_6H_5), 7.78 (m, 2H, C_6H_3), 7.91 (s, 1H, C_6H_3), 8.26 (s, 1H, CH=), 11.49 (bs, 1H, NH). ^{13}C NMR ($CDCl_3$): 69.74, 69.83 ($2C_5H_5$), 68.94, 70.05, 70.49, 70.81 ($2C_5H_4$), 80.03, 80.67 ($2C_{ipsoFc}$), 112.05 (CH=), 118.32, 120.75, 124.93, 128.28 (2C), 130.06 (2C), 132.23 (C_6H_5 + C_6H_3), 125.56, 132.87, 136.45, 138.67, 138.41, 155.32, 197.08 (7C). MS: *m/z* 616 $[M]^+$. *Calcd*. for $C_{36}H_{28}Fe_2N_2O$ (616.28): C 70.16, H 4.58, N 4.54. *Found*: C 70.12, H 4.49, N 4.62%.

1'H,3'H-Spiro[5'-benzoylbenzimidazole-2',3-(1,2-diferrocenylcyclopropene) (5c). Red powder, yield: 0.63 g (17%), (TLC, hexane-CH_2Cl_2, 3:2), m.p.: 211˚C - 212˚C. IR: υ (cm^{-1}) 442, 475, 780, 820, 903, 1001, 1029, 1101, 1242, 1339, 1378, 1404, 1485, 1542, 1560, 1638, 1711, 2889, 3108, 3292, 3462. ^{1}H NMR ($CDCl_3$): 4.21 (s, 5H, C_5H_5), 4.28 (s, 5H, C_5H_5), 4.52 (m, 2H, C_5H_4), 4.61 (m, 2H, C_5H_4), 4.74 (m, 2H, C_5H_4), 4.95 (m, 2H, C_5H_4), 4.79 (bs, 2H, NH), 6.72 (m, 2H, C_6H_5), 7.31 - 7.54 (m, 3H, C_6H_5), 7.71 (d, 1H, C_6H_3, *J* = 6.9 Hz), 7.81 (d, 1H, C_6H_3, *J* = 6.9 Hz), 7.89 (s, 1H, C_6H_3). ^{13}C NMR ($CDCl_3$): 65.98 (C), 66.66, 68.34 ($2C_{ipsoFc}$), 69.66, 69.82

($2C_5H_5$), 68.78, 69.95, 70.42, 70.78 ($2C_5H_4$), 115.56, 125.61, 128.33 (2C), 130.10 (2C), 132.09, 135.02 (C_6H_5 + C_6H_3), 136.67, 138.54, 155.75, 164.73 (4C), 190.14 (C=O). MS: *m/z* 616 $[M]^+$. *Calcd*. for $C_{36}H_{28}Fe_2N_2O$ (616.28): C 70.16, H 4.58, N 4.54. *Found*: C 70.21, H 4.41, N 4.23%.

Cis-4-Aza-2-(1,2-diferrocenylvinyl)-benzimidazole (3d). Orange powder, yield: 1.08 g (35%), (TLC, hexane-CH_2Cl_2, 3:2), m.p.: 168˚C -170˚C. IR: υ (cm^{-1}) 433, 479, 782, 819, 910, 1000, 1023, 1105, 1244, 1343, 1387, 1411, 1480, 1542, 1564, 1838, 2977, 3095, 3295, 3459. ^{1}H NMR ($CDCl_3$): 4.12 (s, 5H, C_5H_5), 4.22 (s, 5H, C_5H_5), 4.26 (m, 2H, C_5H_4), 4.27 (m, 2H, C_5H_4), 4.44 (m, 2H, C_5H_4), 4.51 (m, 2H, C_5H_4), 7.24 (dd, 1H, C_5H_3N, *J* = 5.1, 7.8 Hz), 7.92 (s, 1H, CH=), 8.08 (dd, 1H, C_5H_3N, *J* = 1.5, 7.8 Hz), 8.35 (dd, 1H, C_5H_3N, *J* = 1.5, 5.1 Hz), 11.83 (bs, 1H, NH). ^{13}C NMR ($CDCl_3$): 69.60, 69.91 ($2C_5H_5$), 68.68, 69.77, 70.38, 70.72 ($2C_5H_4$), 80.59, 81.27 ($2C_{ipsoFc}$), 118.77, 126.34, 143.37 (C_5H_3N), 134.93 (CH=), 121.13, 136.11, 147.74, 154.28 (4C). MS: *m/z* 513 $[M]^+$. *Calcd*. for $C_{28}H_{23}Fe_2N_3$ (513.16): C 65.53, H 4.52, N 8.18. *Found*: C 65.32, H 4.42, N 8.12%.

Trans-4-Aza-2-(1,2-diferrocenylvinyl)-benzimidazole (4d). Orange powder, yield: 0.49 g (16%), (TLC, hexane-CH_2Cl_2, 3:2), m.p.: 176˚C - 177˚C. ^{1}H NMR ($CDCl_3$): 4.13 (s, 5H, C_5H_5), 4.19 (s, 5H, C_5H_5), 4.21 (m, 2H, C_5H_4), 4.25 (m, 2H, C_5H_4), 4.46 (m, 2H, C_5H_4), 4.49 (m, 2H, C_5H_4), 7.52 (dd, 1H, C_5H_3N, *J* = 3.3, 5.7 Hz), 7.71 (dd, 1H, C_5H_3N, *J* = 3.3, 5.7 Hz), 7.82 (dd, 1H, C_5H_3N, *J* = 1.5, 5.7 Hz), 8.14 (s, 1H, CH=), 11.54 (bs, 1H, NH). ^{13}C NMR ($CDCl_3$): 69.65, 69.77 ($2C_5H_5$), 68.73, 69.67, 70.15, 70.68 ($2C_5H_4$), 79.51, 80.58 ($2C_{ipsoFc}$), 117.23, 126.46, 145.06 (C_5H_3N), 133.19 (CH=), 121.15, 136.88, 145.63, 154.14 (4C). MS: *m/z* 513 $[M]^+$. *Calcd*. for $C_{28}H_{23}Fe_2N_3$ (513.16): C 65.53, H 4.52, N 8.18. *Found*: C 65.41, H 4.59, N 8.31%.

1'H,3'H-Spiro[4'-azabenzimidazole-2',3-(1,2-diferrocenylcyclopropene)] (5d). Red powder, yield: 0.75 g (24%), (TLC, hexane-CH_2Cl_2, 1:1), m.p.: 211˚C - 212˚C. IR: υ (cm^{-1}) 438, 480, 782, 820, 903, 1001, 1030, 1103, 1242, 1337, 1378, 1402, 1485, 1540, 1561, 1843, 2983, 3099, 3290, 3462. ^{1}H NMR ($CDCl_3$): 4.22 (s, 5H, C_5H_5), 4.27 (s, 5H, C_5H_5), 4.55 (m, 2H, C_5H_4), 4.60 (m, 2H, C_5H_4), 4.62 (m, 2H, C_5H_4), 4.94 (m, 2H, C_5H_4), 4.99 (bs, 2H, NH), 6.76 (dd, 1H, C_5H_3N, *J* = 5.1, 7.2 Hz), 7.43 (d, 1H, C_5H_3N, *J* = 7.2 Hz), 7.84 (d, 1H, C_5H_3N, *J* = 5.1 Hz). ^{13}C NMR ($CDCl_3$): 65.30 (C), 66.61, 68.25 ($2C_{ipsoFc}$), 70.07, 70.15 ($2C_5H_5$), 71.10, 71.71, 72.05, 72.18 ($2C_5H_4$), 135.57, 135.43, 138.52, 154.48 (4C), 113.85, 125.97, 141.19 (C_5H_3N). MS: *m/z* 513 $[M]^+$. *Calcd*. for $C_{28}H_{23}Fe_2N_3$ (513.16): C 65.53, H 4.52, N 8.18. *Found*: C 65.47, H 4.44, N 8.23%.

Cis-5-Aza-2-(1,2-diferrocenylvinyl)-benzimidazole (3e). Orange powder, yield: 1.17 g (38%), (TLC, hexane-CH_2Cl_2, 3:2), m.p.: 166˚C - 167˚C. IR: υ (cm^{-1}) 470, 731, 758, 804, 834, 878, 918, 1001, 1028, 1106, 1242, 1262, 1288, 1371, 1388, 1417, 1440, 1480, 1503, 1598, 1618, 2701, 2909, 2992, 3085. ^{1}H NMR ($CDCl_3$): 4.12 (s, 5H, C_5H_5), 4.19 (s, 5H, C_5H_5), 4.16 (m, 2H, C_5H_4), 4.23 (m, 2H, C_5H_4), 4.45 (m, 2H, C_5H_4), 4.50 (m, 2H, C_5H_4), 7.27 (dd, 1H, C_5H_3N, *J* = 5.1, 7.8 Hz), 7.79 (dd, 1H, C_5H_3N, *J* = 1.2, 7.8 Hz), 7.91 (s, 1H, CH=), 8.08 (dd, 1H, C_5H_3N, *J* = 1.2, 7.8 Hz), 8.35 (dd, 1H, C_5H_3N, *J* = 1.2, 5.1 Hz), 11.90 (bs, 1H, NH). ^{13}C NMR ($CDCl_3$): 69.58, 69.89 ($2C_5H_5$), 68.66, 69.75, 70.38, 70.70 ($2C_5H_4$), 80.58, 81.26 ($2C_{ipsoFc}$), 118.74, 126.35, 143.34 (C_5H_3N), 134.87 (CH=), 121.22, 136.11, 147.75, 154.32 (4C). MS: *m/z* 513 $[M]^+$. *Calcd*. for $C_{28}H_{23}Fe_2N_3$ (513.16): C 65.53, H 4.52, N 8.18. *Found*: C 65.71, H 4.38, N 8.25%.

Trans-5-Aza-2-(1,2-diferrocenylvinyl)-benzimidazole (4e). Orange powder, yield: 0.52 g (17%), (TLC, hexane-CH_2Cl_2, 3:2), m.p.: 174˚C - 176˚C. ^{1}H NMR ($CDCl_3$): 4.14 (s, 5H, C_5H_5), 4.21 (s, 5H, C_5H_5), 4.26 (m, 2H, C_5H_4), 4.27 (m, 2H, C_5H_4), 4.43 (m, 2H, C_5H_4), 4.50 (m, 2H, C_5H_4), 7.22 (dd, 1H, C_5H_3N, *J* = 4.8, 8.1 Hz), 7.81 (dd, 1H, C_5H_3N, *J* = 1.2, 8.1 Hz), 8.13 (s, 1H, CH=), 8.49 (dd, 1H, C_5H_3N, *J* = 1.2, 4.8 Hz), 11.53 (bs, 1H, NH). ^{13}C NMR ($CDCl_3$): 69.64, 69.82 ($2C_5H_5$), 68.73, 69.71, 70.42, 70.63 ($2C_5H_4$), 79.84, 80.25 ($2C_{ipsoFc}$), 117.62, 126.37, 145.11 (C_5H_3N), 136.64 (CH=), 123.05, 137.92, 147.73, 153.29 (4C), MS: *m/z* 513 $[M]^+$. *Calcd*. for $C_{28}H_{23}Fe_2N_3$ (513.16): C 65.53, H 4.52, N 8.18. *Found*: C 65.64, H 4.45, N 8.13%.

1'H,3'H-Spiro[5'-azabenzimidazole-2',3-(1,2-diferrocenylcyclopropene)] (5e). Red powder, yield: 0.65 g (21%), (TLC, hexane-CH_2Cl_2, 1:1), m.p.: 216˚C - 217˚C. IR: υ (cm^{-1}) 433, 479, 782, 819, 910, 1000, 1023, 1105, 1244, 1343, 1387, 1411, 1480, 1542, 1564, 1838, 2977, 3095, 3295, 3459. ^{1}H NMR ($CDCl_3$): 4.13 (s, 5H, C_5H_5), 4.16 (s, 5H, C_5H_5), 4.58 (m, 2H, C_5H_4), 4.69 (m, 2H, C_5H_4), 4.84 (m, 2H, C_5H_4), 4.98 (m, 2H, C_5H_4), 4.95 (bs, 2H, NH), 6.61 (d, 1H, C_5H_3N, *J* = 7.5 Hz), 7.07 (d, 1H, C_5H_3N, *J* = 7.5 Hz), 7.82 (s, 1H, C_5H_3N). ^{13}C NMR ($CDCl_3$): 65.32 (C), 66.54, 67.97 ($2C_{ipsoFc}$), 69.85, 69.93 ($2C_5H_5$), 70.63, 70.78, 71.45, 71.98 ($2C_5H_4$), 129.74, 130.56, 138.12, 153.86 (4C), 120.52, 141.42, 147.51 (C_5H_3N). MS: *m/z* 513 $[M]^+$. *Calcd*. for $C_{28}H_{23}Fe_2N_3$ (513.16): C 65.53, H 4.52, N 8.18. *Found*: C 65.62, H 4.37, N 8.15%.

Cis-8-(1,2-Diferrocenylvinyl)purine (3f). Orange powder, yield: 1.23 g (40%), (TLC, hexane-CH_2Cl_2, 3:2), m.p.: 182˚C - 183˚C); IR: υ (cm^{-1}) 475, 494, 733, 792, 811, 916, 999, 1028, 1040, 1107, 1240, 1295, 1338, 1388,

1413, 1502, 1591, 1615, 2908, 2990, 3089, 3422. ^{1}H NMR ($CDCl_3$): 4.13 (s, 5H, C_5H_5), 4.23 (s, 5H, C_5H_5), 4.28 (m, 2H, C_5H_4), 4.32 (m, 2H, C_5H_4), 4.48 (m, 2H, C_5H_4), 4.50 (m, 2H, C_5H_4), 7.98 (s, 1H, CH=), 8.96 (s, 1H, $C_4H_2N_2$), 9.15 (s, 1H, $C_4H_2N_2$), 11.97 (bs, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 69.69, 69.98 ($2C_5H_5$), 68.94, 70.22, 70.33, 70.91 ($2C_5H_4$), 80.03, 80.89 ($2C_{ipsoFc}$), 136.82 (CH=), 120.03, 134.73, 146.18, 155.44 (4C), 146.58, 151.97 ($C_4H_2N_2$). MS: *m/z* 514 $[M]^+$. *Calcd*. for $C_{27}H_{22}Fe_2N_4$ (514.15): C 63.07, H 4.32, N 10.89. *Found*: C 63.02, H 4.25, N 10.93%.

Trans-8-(1,2-Diferrocenylvinyl)purine (4f). Orange powder, yield: 0.55 g (18%), (TLC, hexane-CH_2Cl_2, 3:2), m.p.: 191˚C - 192˚C. ^{1}H NMR ($CDCl_3$): 4.14 (s, 5H, C_5H_5), 4.21 (s, 5H, C_5H_5), 4.18 (m, 2H, C_5H_4), 4.30 (m, 2H, C_5H_4), 4.36 (m, 2H, C_5H_4), 4.47 (m, 2H, C_5H_4), 8.25 (s, 1H, CH=), 8.94 (s, 1H, $C_4H_2N_2$), 9.12 (s, 1H, $C_4H_2N_2$), 11.78 (bs, 1H, NH). ^{13}C NMR ($CDCl_3$): 69.80, 69.86 ($2C_5H_5$), 69.04, 70.28, 70.52, 71.05 ($2C_5H_4$), 79.11, 80.03 ($2C_{ipsoFc}$), 137.22 (CH=), 120.41, 134.70, 145.34, 153.16 (4C), 146.18, 151.82 ($C_4H_2N_2$). MS: *m/z* 514 $[M]^+$. *Calcd*. for $C_{27}H_{22}Fe_2N_4$ (514.15): C 63.07, H 4.32, N 10.89. *Found*: C 63.05, H 4.22, N 10.81%.

7'H,9'H-Spiro[purine-8',3-(1,2-diferrocenylcyclopropene)] (5f). Red powder, yield: 0.71 g (23%), (TLC, hexane-CH_2Cl_2, 1:1), m.p.: 206˚C - 208˚C. IR: υ (cm^{-1}) 484, 782, 825, 903, 976, 1002, 1030, 1106, 1243, 1336, 1379, 1406, 1485, 1538, 1559, 1612, 1855, 2157, 2826, 3096, 3146, 3291, 3457. ^{1}H NMR ($CDCl_3$): 4.28 (s, 5H, C_5H_5), 4.285 (s, 5H, C_5H_5), 4.60 (m, 2H, C_5H_4), 4.64 (m, 2H, C_5H_4), 4.66 (m, 2H, C_5H_4), 4.93 (m, 2H, C_5H_4), 5.45 (bs, 2H, NH), 8.26 (s, 1H, $C_4H_2N_2$), 8.38 (s, 1H, $C_4H_2N_2$). ^{13}C NMR ($CDCl_3$): 64.88 (C), 65.88 ($2C_{ipsoFc}$), 70.13, 70.15 ($2C_5H_5$), 71.15, 72.04, 72.36, 72.43 ($2C_5H_4$), 134.63, 135.64, 138.79, 158.81 (4C), 142.91, 152.57 ($C_4H_2N_2$). MS: *m/z* 514 $[M]^+$. *Calcd*. for $C_{27}H_{22}Fe_2N_4$ (514.15): C 63.07, H 4.32, N 10.89. *Found*: C 62.98, H 4.36, N 10.78%.

2.4. Determination of the Crystal Structure

The unit cell parameters and the X-ray diffraction intensities were recorded on a on a Gemini diffractometer (detector Atlas CCD, Cryojet N_2). The structure of compound 3b was solved by the direct method (SHELXS-97 [31]) and refined using full-matrix least-squares on F^2.

Crystal data for $C_{30}H_{26}Fe_2N_2$(3b): M = 526.23 $gmol^{-1}$, monoclinic P21/c, a = 11.1977(4), b = 16.8081(6), c = 25.4637(10) Å, α = 90, β = 97.128(3), γ = 90˚, V = 4755.5(3) $Å^3$, T = 130(2) K, Z = 8, ρ = 1.470 Mg/m^3, wavelength 0.71073 Å, F(000) = 2176, absorption coefficient 1.242 mm^{-1}, index ranges $-11 \le h \le 13$, $-20 \le k \le 17$, $-21 \le l \le 31$, scan range $3.42^\circ \le \theta \le 25.68^\circ$, 9012 independent reflections, R_{int} = 0.0375, 21892 total reflections, 615 refinable parameters, final R indices [$I > 2\sigma(I)$] R_1 = 0.0408, wR_2 = 0.0960, R indices (all data) R_1 = 0.0596, wR_2 = 0.1042, goodness-of-fit on F^2 1.042, largest difference peak and hole 0.509/−0.368 $eÅ^{-3}$.

CCDC 981930 contains the supplementary crystallographic data for this paper (compound 3b). These data can be obtained free of charge at www.ccdc.cam.ac.uk/const/retrieving.html [or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge DB2 1EZ, UK; fax: (internet.) +44 1223/336 033; E-mail: deposit@ccdc.cam.ac.uk].

2.5. Cytotoxicity Assay

The compounds were screened *in vitro* against human cancer cell lines HCT-15 (human colorectal adenocarcinoma), MCF-7 (human mammary adenocarcinoma), K562 (human chronic myelogenous leukemia), U251 (human glioblastoma), PC-3 (human prostatic adenocarcinoma), SKLU-1 (human lung adenocarcinoma). The cell lines were supplied by the National Cancer Institute (USA). The human tumor cytotoxicity was determined using the protein-binding dye sulforhodamine B (SRB) in the microculture assay to measure the cell growth, as is described in the protocols established by the NCI [32] [33]. The cell lines were cultured in the RPMI-1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 10,000 units/ml penicillin G sodium, 10 μg/ml streptomycin sulfate, 25 μg/ml amphotericin B (Gibco) and 1% non-essential amino acids (Gibco). The cultures were maintained at 37˚C in a humidified 5% CO_2 atmosphere. As was determined using trypan blue, the viability of the cells used in the experiments exceeded 95%.The cells were removed from the tissue culture flasks by treatment with trypsin and diluted with fresh media. 100-ml cell suspension aliquots, containing 5000 - 10,000 cells per well, were transferred to 96-well microtiter plates (Costar) and incubated at 37˚C for 24 h in a 5% CO_2 atmosphere.

Stock solutions of the test compounds initially dissolved in DMSO (20 mM) were prepared and further diluted in the medium to produce the desired concentrations. 100-ml aliquots of the diluted solutions of the test

compounds were added to each well. The cultures were exposed to the compound at concentrations 50 μM for 48 h. After the incubation period, the cells were fixed to a plastic substratum by the addition of 50 μl of cold 50% aqueous trichloroacetic acid. The plates were incubated at 4˚C for 1 h, washed with tap H_2O, and air-dried. The cells fixed with trichloroaceticacid were stained by the addition of 0.4% SRB. Free SRB solution was removed by washing with 1% aqueous acid acetic. The plates were air-dried, and the bound dye was solubilized by the addition of 100 μL of 10 mM unbuffered tris base. The plates were placed on a shaker for 5 min prior to analysis. The optical densities were determined using an Ultra Microplated Reader (El_x 808: Bio-Tek Instruments, Inc., Winooski, VT, USA) at a test wavelength of 515 nm.

3. Results and Discussion

3.1. Chemistry

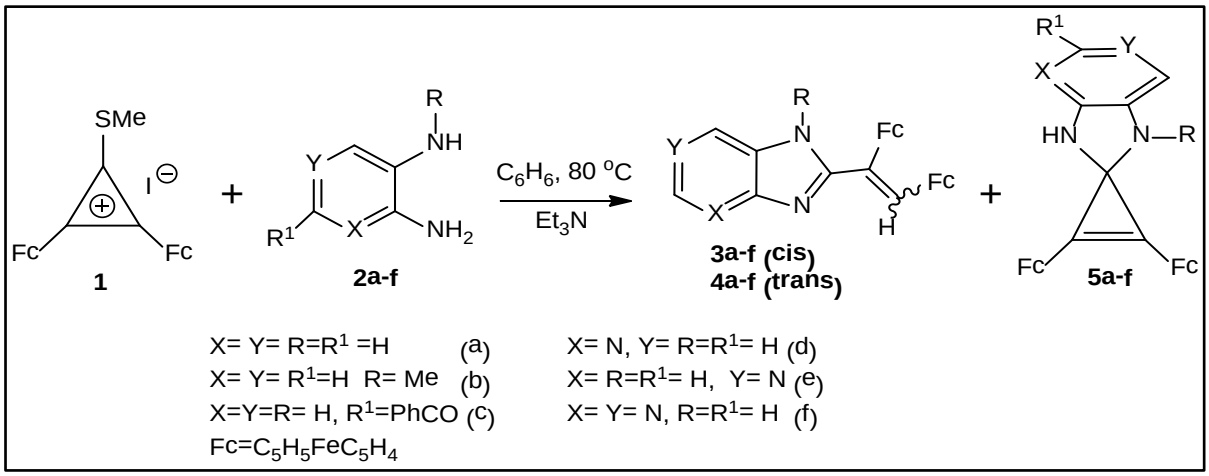
In this paper, we study the interaction of 2,3-diferrocenyl-1-methylsulfanylcyclopropenylium iodide 1 [4] with aromatic carbo- and heterocyclic *o*-diamines 2a-f. We have found that salt 1 reacts with *o*-phenylenediamines 2a-c upon boiling in dry benzene in the presence of Et_3N (~2 - 3 h), forming *cis*- and *trans*-2-(1,2-diferrocenylvinyl) imidazoles 3a-c (~35% - 40%) and 4a-c (~10% - 15%) and spiro[benz-imidazole-2',3-(1,2-diferrocenylcyclopropenes)] 5a-c (~20% - 30%) (**Scheme 1**).

The products of each reaction were separated using Al_2O_3 (activity grade III) column chromatography. The structures of the compounds were established using IR, ^1H and ^{13}C NMR spectroscopy, mass spectrometry and elemental analysis. For example, the ^1H NMR spectra of compounds 3a-c and 4a-c contain the characteristic signals from protons of the ferrocenyl, aryl, and methyl groups, as well as the singlets of protons from the -NH and =CH fragments. An important feature of the ^1H NMR spectra of compounds 3a-c is the fact that they contain signals from the hydrogen atom of the CH = fragment in a stronger field (δ = 7.93, 6.56, 8.12) than the corresponding signals in compounds 4a-c (δ = 8.16, 6.71, 8.26).

The benzimidazole structure is additionally confirmed by the fact that the ^{13}C NMR spectra contain the corresponding numbers of signals from the quaternary carbons, as well as from the CH=, CH_3, C_6H_4, C_6H_3, C_6H_5, and 2 Fc groups.

To establish the geometrical configuration of compounds 3a-c, we performed X-ray diffraction analysis of single crystals of 3b, which were isolated via crystallization from CH_2Cl_2. The general form of molecule 3b is shown in **Figure 1**, and the main geometrical parameters of compound 3b are listed in **Table 1**.

The X-ray findings show that the structure of 3b is that of *cis*-2-(1,2-diferrocenylvinyl)-1-methylbenzimidazole. By analogy with it, compounds 3a and 3c were also regarded as having the *cis*-configuration. Hence, compounds 4a-c contain the 1,2-diferrocenylvinyl fragment with the *trans*-orientation of the ferrocenyl groups. A characteristic feature of the crystal structure of 3b is the presente of two molecules in the unit cell differing in the orientation of the four ferrocenyl substituents.



Scheme 1. Reaction of 2,3-diferrocenyl-1-methylsulfanil-cyclopropenylium iodide 1 with aromatic carbo- and heterocyclic diamines 2a-f.

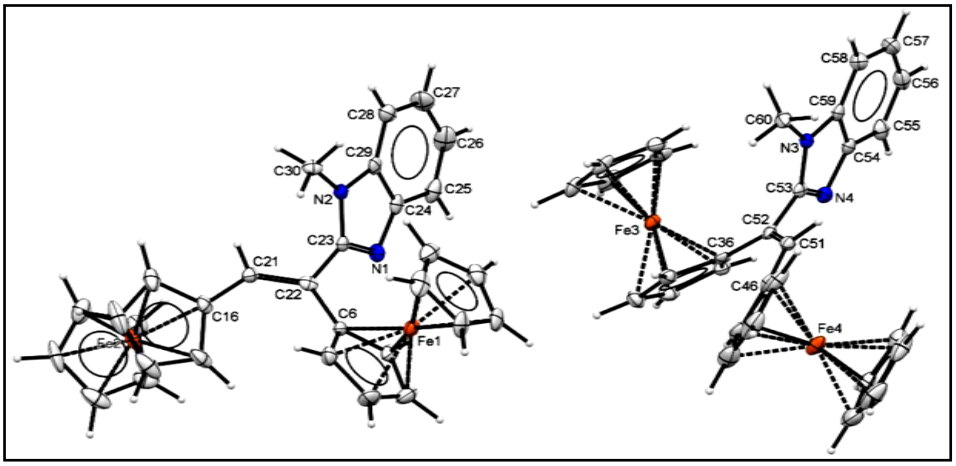


Figure 1. Molecular structure of 3b.

The structures of compounds 5a-c were established using IR, ^{1}H and ^{13}C NMR spectroscopy, mass spectrometry, and elemental analysis. For example, the ^{1}H NMR spectrum of compound 5a characterizes it as a molecule with a symmetrical structure, whose spectrum contains one signal from the protons of two C_5H_5 rings and two signals from the protons of two C_5H_4 fragments of two ferrocene sandwiches, as well as two signals from the protons of the *o*-phenylene ring and one singlet from the protons of two NH groups. The ^{13}C NMR spectrum of compound 5a contains one signal from the two C_{ipso} Fc carbons (δ = 66.74), one from the quaternary C_{spiro} carbon atom (δ = 65.15), and two signals from four C_{ipso} carbon atoms (δ = 130.03, 137.05). The data of the ^{1}H and ^{13}C NMR spectra of compounds 5b and 5c are provided in the Experimental section. The IR spectra of compounds 5a-c contain bands at 1828 - 1835 cm^{-1}, which are characteristic of the cyclopropenyl group. Taken as a whole, these spectral data confirm the structures of compounds 5a-c to be those of 1'H,3'H(or Me)-spiro[benzimidazoline-2',3-(1,2-diferrocenylcyclopropenes)].

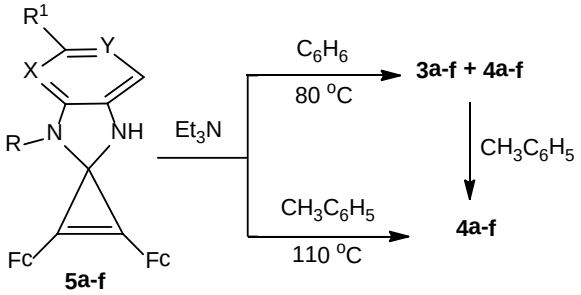
Spiranes 5a-c are fine crystals of red color, stable upon storage at room temperature (20˚C - 25˚C) in the inert atmosphere. At an elevated temperature or upon exposure to air, they decompose via the opening of the three-carbon cyclopropene ring and form a mixture of benzimidazoles 3a-c and 4a-c, with the amount of the *trans*-isomer 4a-c in the mixture increasing over time.

On the basis of these preliminary data, we studied the behavior of compounds 3a-c, 4a-c, and 5a-c upon heating. It turned out that boiling of spiroimidazolines 5a-c in benzene (~3 - 4 h) leads to the formation of a mixture of *cis*-3a-c and *trans*-4a-c benzimidazoles (yield ~85%, ~2:1); subsequent boiling of the resultant mixture in toluene yields the pure *trans* isomer 4a-c (~71%). *Trans*-4a-c are also formed as a result of direct boiling of spiranes 5a-c in toluene (~4 - 5 h, ~77%) (**Scheme 2**).

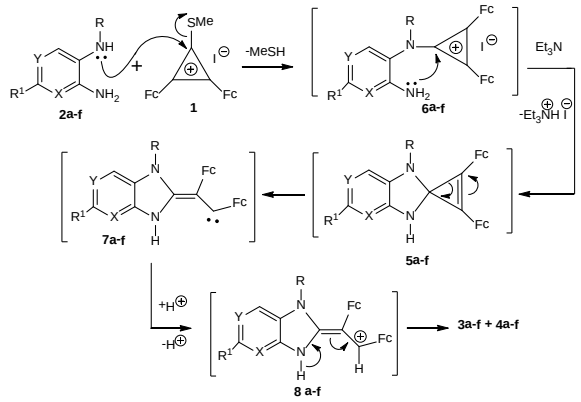
Further, we have found that diferrocenylcyclopropenylium iodide 1 reacts with 2,3- and 3,4-diaminopyridines 2d,e and 4,5-diaminopyrimidine 2f under similar conditions upon boiling in benzene for a longer time (~4 - 6 h), forming the following products: bicyclic aza- and diazabenzimidazoles (*cis*-3d-f and *trans*-4d-f, ~3:1), as well as spirane azabenzimidazoles 5d,e and diazabenzimidazole 5f, *i.e.*, 7'H,9'H-spiro[purine-8',3-(1,2-diferrocenylcyclopropene)] (~31%) (**Scheme 1**). The structures of all chromatographically separated products (Al_2O_3, grade III) were established and completely confirmed using the IR, ^{1}H and ^{13}C NMR spectra, mass spectrometry, and elemental analysis. It has also been found that spiro aza- and diazabenzimidazoles 5d-f upon heating in benzene (~10 - 15 h) are transformed into bicyclic aza- and diazabenzimidazoles 3d-f and 4d-f (**Scheme 2**); upon boiling in toluene, compounds 5d-f and 3d-f are transformed into *trans*-4d-f (**Scheme 2**).

The presumable mechanism describing the formation of the derivatives of 2-(1,2-diferrocenylvinyl)benz- and azabenzimidazoles 3a-e and 4a-e, 8-(1,2-diferrocenylvinyl)purines 3f and 4f, as well as spiranes 5a-f, is shown in **Scheme 3**.

One of the nitrogen atoms of 1,2-diamines 2a-f first attacks atom C-1 of 2,3-diferrocenyl-1 methylsulfanylcyclopropenylium iodide 1 and substitutes the MeS group with the formation of 1-aminocyclopropenylium cations 6a-f. The repeated attack of another nitrogen atom on the C-1 carbon atom in cations 6a-f yields the spirane benz- and azabenzimidazoles 5a-f. The opening of the small cycle in the cyclopropene fragments of spi-



Scheme 2. Termal intramolecular transformation of spiro (imidazoline-cyclo-propenes) 5a-f and *cis*-2-(1,2-diferrocenylvinyl)benz- and azabenzimidazoles 3a-f into trans-imidazoles 4a-f.



Scheme 3. Presumable mechanism of formation of compounds 5a-f, 3a-e and 4a-e.

ranes 5a-f with the formation of vinylcarbene intermediates 7a-f and subsequent intramolecular transformation of the carbenes lead to the formation of bicyclic imidazoles 3a-f and 4a-f.

3.2. Electrochemistry

Figure 2 shows the typical cyclic voltammogram of compound 3a. One can observe two oxidation signals (I_a and II_a) with the corresponding reduction signals (I_c and II_c). For process I, the resultant anodic and cathodic peak potentials $E_{pa}(I)$ and $E_{pc}(I)$ were −0.033 V/Fc-Fc$^+$ and 0.0259 V/Fc-Fc$^+$, respectively. On the other hand, the corresponding potentials $E_{pa}(II)$ and $E_{pc}(II)$ for process II were 0.130 and 0.189 V/Fc-Fc$^+$. For both processes, a ΔE_p value of 0.059 V was estimated, without any dependence on the scan rate. The peak current values I_p adhered to a linear relationship with $v^{1/2}$. Therefore, two-step reversible oxidation of the ferrocene moieties for processes I and II was suggested. The formal electrode potential was evaluated as the half-sum of the anodic and cathodic peak potentials, $E^{0'} = (E_{pa} + E_{pc})/2$ [30]. Its values for processes I and II were $E^{0'}(I)$ = 0.003 V/Fc-Fc$^+$ and $E^{0'}(II)$ = 0.159 V/Fc-Fc$^+$. These values enabled us to estimate the comproportionation constant $K_{com} = 4.32 \times 10^2$ [34]-[36]. The electrochemical response of compounds 3b and 3d is very similar to that observed for compound 3a, although there are slight changes in the values of the formal electrode potentials for processes I and II. In the case of compound 5f, a different electrochemical response was observed.

The typical cyclic voltammogram of compound 5f presents two oxidation signals (I_a and I^*) and one reduction process (I_c), see **Figure 3**. When the scan rate was increased, an increase in the current values was detected for all the signals. A linear relationship between I_p and $v^{1/2}$, characteristic for reversible electrochemical processes, was observed for I_a and I_c. These results, as well as the absence of the corresponding reduction signal for I^*, indicate that I^* is associated with an adsorption-desorption process; meanwhile, I_a and I_c are attributed to the electron transfer for ferrocene moieties. The difference between the potential peak values ΔE_p = 0.10 V and the for-

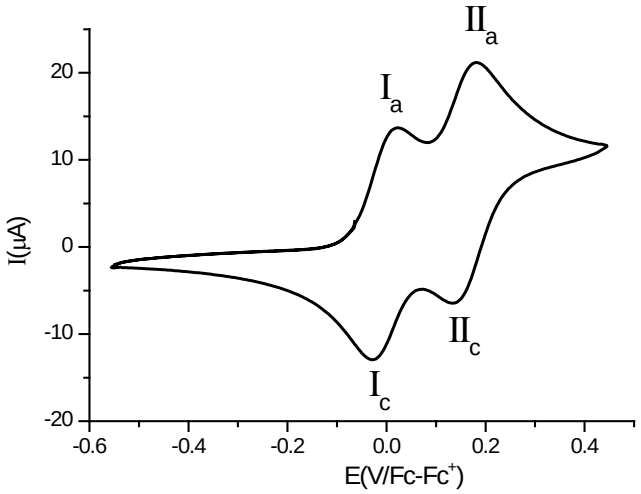


Figure 2. Cyclic voltammogram obtained for 3a in the presence of 0.1 M TBAPF6 in CH3CN. Scan rate 0.1 V/s, platinum working electrode.

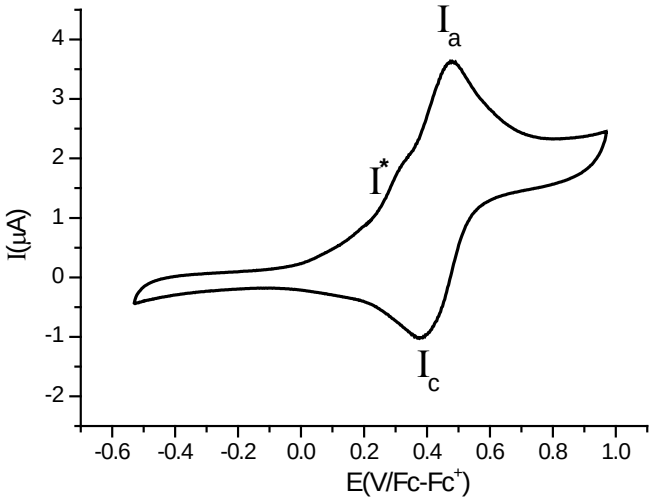


Figure 3. Cyclic voltammogram obtained for 5f in the presence of 0.1 M TBAPF6 in CH3CN. Scan rate 0.1 V/s, platinum working electrode.

mal electrode potential $E^{0'}(I) = 0.426$ V/Fc-Fc$^+$ were calculated.

According to literature, the difference between the formal electrode potentials $\Delta E(II\text{-}I) = 0.073$ V was evaluated for the E_r E_r mechanism using the working curve ΔE_p vs. ΔE [33] [34]. On the basis of this value, it was possible to calculate the formal electrode potential for process II: $E^{0'}(II) = 0.353$ V/Fc-Fc$^+$. **Table 2** summarizes the electrochemical behaviour of all the compounds studied in this work.

The estimated values of K_{com} for all the compounds suggest that the electronic charge is slightly delocalized in the electrochemically generated mixed valence, according to the Robin-Day classification [35] [36]. For compound 5f, the lowest electronic communication in the electrochemically generated mixed-valence species was observed. It can be noticed that the presence of different substituents around each ferrocene moiety in compounds 3a, 3b and 3d makes a considerable contribution to each formal electrode potential, and therefore the K_{com} values are increased because of this effect.

3.3. Pharmacology

In order to examine the applicability of two types of compounds (3a, 3c-f and 5d, 5f) as antitumor agents, they

were tested *in vitro* against six human tumor cell lines: U-251 (glioma), PC-3 (prostate cancer), K-562 (leukemia), HCT-15 (colon cancer), MCF-7 (breast cancer) and SKLU-1 (lung cancer). Primary screening at a fixed concentration showed cytotoxicity against the six tested human tumor cell lines and besides against human lymphocytes (MT2). Cisplatin at the same concentration was used as the positive control. The compounds were used as 50 µM solutions in DMSO (**Table 3**).

Compound 5f showed 100% inhibition of cell growth at 50 µM for five human tumor cell lines, 86.8% inhibition for SKLU-1, and also 90.8% inhibition for human lymphocytes (MT2). Compound 3f showed higher activity than cisplatin for U-251 and MCF-7 (**Table 3**).

4. Conclusion

Twelve novel 2-(1,2-diferrocenylvinyl)benz- and azabenzimidazoles 3a-f and 4a-f and six novel spiro-[(aza)-benzimidazoline-2',3-(1,2-diferrocenylcyclopropenes)] 5a-f were synthesized and structurally characterized using spectroscopic techniques. Seven of these synthesized compounds displayed modest cytotoxic activity in the micromolar range. Compound 5f, which contains the purinyl moiety, appeared to be the most active against six human tumor cell lines. Compounds 3b and 3f exhibited the highest activity against five and two human tumor cell lines, respectively, vs. cisplatin, which was used as the reference. These results identified 7'H,9'H-spiro[purine-8',3-(1,2-diferrocenylcyclopropene)] 5f, *cis*-2-(1,2-diferrocenylvinyl)-1-methyl-benzimidazole 3b and *cis*-8-(1,2-diferrocenylvinyl) purine 3f as new possible candidates for antitumor chemotherapy. The study suggests that the potential of these candidates needs to be further explored in order to discover and develop better and yet safer therapeutic antitumor agents.

Table 2. The formal electrode potentials E^0(I), E^0(II) and the K_{com} values for compounds 3a, 3b, 3d and 5f.

Compound	E^0(I)	E^0(II)	ΔE^0(II-I)	K_{com}
3a	0.003	0.159	0.156	4.32 × 102
3b	0.079	0.236	0.157	4.50 × 102
3d	−0.036	0.130	0.166	6.38 × 102
5f	0.426	0.353	−0.073	0.058

The formal electrode potential ($E^{0'}$) vs. Fc/Fc^+ in the presence of 0.1 M $TBAPF_6$-CH_3CN.

Table 3. Inhibition of the growth (%) of human tumor cell lines and human lymphocytes (MT2) cell for compounds 3a, b, c-f, 5d, f at 50 µM in DMSO.

Compounds	% of growth inhibition						
	U-251	PC-3	K562	HCT-15	MCF-7	SKLU-1	MT2
3a	24.48	29.4	NA	24.1	13.1	33.7	78.0
3b	80.11	88.89	86.08	78.69	96.59	58.73	NA
3c	69.4	53.0	NA	NA	41.68	42.92	NA
3d	36.04	45.8	NA	4.7	1.7	13.2	61.97
3e	41.4	36.5	NA	NA	1.9	NA	62.4
3f	91.8	55.3	NA	33.2	80.2	57.7	51.8
5d	55.45	67.3	NA	NA	7.9	3.1	69.4
5f	100	100	100	100	100	86.8	90.8
Cisplatin	89.9	86.7	74.4	81.8	77.9	95.8	22.9

NA: not active.

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