Nitro-imidazoles in ferrocenyl alkylation reaction. Synthesis, enantiomeric resolution and *in vitro* and *in vivo* bioeffects

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ARTICLE INFO

ABSTRACT

Ferrocenylalkyl nitro-imidazoles (**4a-h**, **5a-h**) were prepared *via* the regiospecific reaction of the α-(hydroxy)alkyl ferrocenes, FcCHR (OH) (**1a–h**; Fc = ferrocenyl; R = H, Me, Et, Pr, *i*-Pr, Ph, *ortho*-Cl-Ph, *ortho*-I-Ph), with nitro-imidazoles in aqueous organic medium (H_2O-CH_2Cl_2) at room temperature in the presence of HBF_4, within several minutes in good yields. X-ray structural data for racemic (*R,S*)-1-*N*-(benzyl ferrocenyl)-2-methyl-4-nitroimidazole (**5f**) were determined. The resulting enantiomers were resolved into enantiomers by analytical HPLC on modified amylose or cellulose chiral stationary phases. The viabilities of **4b**, **4d**, **5b**, **5c** *in vitro*, and in experiments *in vivo* antitumor effects of 1-*N*-ferrocenyl-lethyl-4-nitroimidazole (**4b**) against murine solid tumor system Ca755 carcinoma were evaluated.

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1. Introduction

The conjugation of nitrogen-containing hetero cycles with ferrocene represents perspective directions for design and synthesis of bioactive compounds with different types of activities including antianemic, tuberculostatic, antimalarial, antimicrobic and antiproliferative [1–10]. The antiproliferative activity has been especially investigated by numerous research groups [10–15].

Among heterocycles, azoles and especially imidazole cause a high interest because represent essential components of DNA, RNA, histidine amino acid or drugs [10–14,16–20]. On the other hand, such nitrogen-heterocycle–ferrocene ensembles possess positive enthalpies of formation to give highly energetic structures [21]. Well known, that both ferrocenes and nitro-compounds were applied for solid rocket propellant in the early 1970s [10]. Moreover, nitro groups being in the structures of nitro-glycerin and trinitro-toluene make these compounds high explosive. To our knowledge, there are no systematic studies on synthesis of compounds combining both nitro-imidazole and ferrocene fragments excluding the data in the review article of Tverdohlebov with co-authors [21].

Ferrocene-modification of organic compounds was widely represented in reviews [17,22,23] and special recent issue [24] The ferrocenylalkylation method for the introduction of ferrocenylalkyl groups into various nucleophilic substrates was based on the substitution reactions of ferrocenylalkyl amines or α-(hydroxy)alkyl ferrocenes with nucleophiles [22]. The later one is extensively explored approach to ferrocene-based compounds [23,25,26].

Recently, for example, Moiseev and co-workers elaborated new synthetic route to ferrocenes of such type through unstable α-ferrocenylalkyl carbonates under neutral conditions [27]. Some more original pathway for modified ferrocenes through the direct C–H functionalization of aromatics by the C–C coupling of halogen-free (hetero)arenes with lithium ferrocenes allows the preparing of planar chiral ferrocenes [28].

Other well-known pathway to ferrocene heterocyclic molecules is consists in formation of carbonyl-connected ferrocene-C(O)-heterocycle through a dehydrohalogenation reaction between ferrocenoyl chloride and NH-heterocycle [29]. Redox amination of ferrocenyl ketones and formylferrocene with pyrroline allows preparing ferrocene-substituted pyrroles [30].

In this paper, the development of our ferrocene-devoted works is reported [11–13,31–34]. Early we proposed a simple route to ferrocene-based compounds [35]. Moreover, using this protocol, enantiomeric-enriched ferrocene-based compounds were prepared recently [36]. Herein, according to this synthetic approach a series of unknown ferrocene-containing nitro-imidazoles was prepared in good yields. All synthesized compounds were characterized by ^{1}H, ^{13}C NMR, EI-MS and microanalytical data. For compound **5f** X-ray structural data were obtained. A series of racemic (*S,R*)-ferrocene nitro-imidazoles (14 pairs of ferrocene-based compounds) was separated into enantiomers by analytical HLPC on chiral modified amylose. The antitumor effects were evaluated for compound **4b** against Ca755 carcinoma experimental tumor model. The index of tumor growth inhibition (TGI) amounting to 22% (in comparison with controls) was determined for compound **4b** in daily dose equal to 10 mg kg^{-1}. At the dose of 20 mg kg^{-1} the tested compound **4b** was not effective against Ca755 carcinoma. On the contrary, a stimulation effect equal to 46% was observed in this case. Cytotoxicities were evaluated by MTT test for compounds **4b**, **4d**, **5b**, **5c**, and for compound **4b** maximal tolerated dose was found in *in vivo* experiments.

2. Experimental

2.1. Starting materials and analytical instrumentations

The starting ferrocenyl methanol (**1a**) was obtained from trimethylferrocenyl methylammonium iodide according to a well-known procedure [37]. Others ferrocenyl alcohols (**1b-h**) were synthesized from ferrocene by acylation with the corresponding acid chlorides according to the Friedel–Crafts procedure and subsequent reduction either by sodium borohydride in water [38] or by lithium aluminum hydride in diethyl ether or THF [12,39]. 4 (5)-Nitroimidazole (**2a**) and 2-methyl-4 (5)-nitroimidazole (**2b**) were prepared according to patents [40,41]. Syntheses were carried out in a 100 mL flask with fluoroplastic propeller mixer and thermometer. Sulfuric acid 92% (66.3 g) and 10.4 g (0.15 mol) imidazole or 2-methyl-4 (5)-nitroimidzole (**2b**) were added into the flask with stirring. The temperature was raised to 145 °C, then, 29.8 g (0.35 mol) sodium nitrate was added in portions to the mixture. After stirring at 145 °C during 6 h cold water (100 mL) was added to the flask and the mixture was cooled to the room temperature. The crud products were isolated by 25% ammonia solution to pH 10, filtered and dried by lyoplilization at −52 °C and 0.27 mbar for 16 h. Both imidazoles represented white powders, yield **2a** 75%, m. p. 306–310 °C; yield **2b** 95%, m. p. 253–256 °C.

All solvents were purified and dried by standards techniques. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance instrument at 400 MHz for protons and 100 MHz for carbon respectively. Chemical shifts are given in ppm relative to solvent residual protons. EI mass spectra were taken on a Finnigan Polaris Q spectrometer at 70 eV and the temperature of the ion chamber 250 °C.

IR spectra were recorded on a UR-20 (Karl Zeiss) spectrophotometer.

2.2. General procedure

To a mixture of 1.0 mmol of ferrocene alcohol and 1.0 mmol of the corresponding nitroimidazole in 1.0 ml of methylene dichloride, 0.18 ml of 45% aqueous solution of fluoroboric acid was added under vigorous stirring. The agitation was continued for 5 min then diethyl ether (15 ml), the same amount of cold water, and 5–10 mg of ascorbic acid were added to the reaction flask. After vigorous shaking of the mixture, the organic solution was separated, washed with cold water (3 × 15 ml), the solvents were removed *in vacuo*, and the residue was dried over CaCl$_2$ in a desiccator.

2.2.1. 4-Nitro-1-(ferrocenylmethyl)-1H-imidazole (**4a**)

Yield 64%. Orange crystals, m. p. 206 °C. EI-MS, *m/z* (RI, %) 311 [M$^+$] (67). ^{1}H NMR (CDCl$_3$, δ, ppm): 4.21 (s, 5H, Fc); 4.25 (s, 2H, Fc); 4.28 (s, 2H, Fc); 4.95 (s, 2H, CH$_2$); 7.41 (s, 1H, Im(C-2)); 7.70 (s, 1H, Im(C-5)). ^{13}C NMR (CDCl$_3$, δ, ppm): 48.28 (CH$_2$); 68.89 (C$_5$H$_4$); 68.99 (C$_5$H$_5$); 69.64 (C$_5$H$_4$); 79.80 (C$_5$H$_4$ -ipso); 118.76 (Im(C-5)); 135.22 (Im(C-2)); 148.37 (Im(C-NO$_2$)). Anal.: C 53.99; H 4.17; N 13.48%. Calc. for C$_{14}$H$_{13}$FeN$_3$O$_2$: C, 54.05; H, 4.21; N, 13.51%.

2.2.2. 4-Nitro-1-(1-ferrocenylethyl)-1H-imidazole (**4b**)

Yield 65%. Yellow crystals, m. p. 106 °C. EI-MS, *m/z* (RI, %) 325 [M$^+$] (25). ^{1}H NMR (CDCl$_3$, δ, ppm): 1.89 (d, J = 6.9 Hz, 3H, CH$_3$); 4.14 (s, 1H, Fc); 4.19 (s, 5H, Fc); 4.26 (s, 1H, Fc); 4.28 (s, 2H, Fc); 5.25 (q, J = 6.9 Hz, 1H, CH); 7.42 (s, 1H, Im(C-2)); 7.69 (s, 1H, Im(C-5)). ^{13}C NMR (CDCl$_3$, δ, ppm): 21.95 (CH$_3$); 54.82 (CH); 65.79 (C$_5$H$_4$); 67.95 (C$_5$H$_4$); 68.78 (C$_5$H$_4$); 69.12 (C$_5$H$_5$); 69.40 (C$_5$H$_4$); 86.35 (C$_5$H$_4$-ipso); 117.76 (Im(C-5)); 134.50 (Im(C-2)); 147.45 (Im(C-NO$_2$)). Anal.: C 55.43; H 4.54; N 12.94. Calc. for C$_{15}$H$_{15}$FeN$_3$O$_2$: C, 55.41; H, 4.65; N, 12.92%.

2.2.3. 4-Nitro-1-(1-ferrocenylpropyl)-1H-imidazole (**4c**)

Yield 50%. Red-brown crystals, m. p. 87 °C. EI-MS, *m/z* (RI, %) 339 [M$^+$] (34). ^{1}H NMR (CDCl$_3$, δ, ppm): 0.95 (t, J = 7.4 Hz, 3H, CH$_3$); 2.04 (m, 1H, CH$_2$); 2.36 (m, 1H, CH$_2$); 4.07 (s, 1H, Fc); 4.15 (s, 5H, Fc); 4.23 (s, 1H, Fc); 4.26 (s, 2H, Fc); 4.88 (dd, J = 10.8 Hz, 3.8, 1H, CH); 7.47 (s, 1H, Im(C-2)); 7.72 (s, 1H, Im(C-5)). ^{13}C NMR (CDCl$_3$, δ, ppm): 11.19 (CH$_3$); 29.04 (CH$_2$); 61.61 (CH); 66.30 (C$_5$H$_4$); 67.14 (C$_5$H$_4$); 68.53 (C$_5$H$_4$); 69.07 (C$_5$H$_5$); 69.15 (C$_5$H$_4$); 86.96 (C$_5$H$_4$-ipso); 117.73 (Im (C-5)); 135.10 (Im (C-2)); 147.98 (Im (C- NO$_2$)). Anal.: C 56.68; H 5.05; N 12.41. Calc. for C$_{16}$H$_{17}$FeN$_3$O$_2$: C, 56.66; H, 5.05; N, 12.39%.

2.2.4. 4-Nitro-1-(1-ferrocenylbutyl)-1H-imidazole (**4d**)

Yield 48%. Red-brown crystals, m. p. 111 °C. EI-MS, *m/z* (RI, %) 353 [M$^+$] (29). ^{1}H NMR (CDCl$_3$, δ, ppm): 0.98 (t, J = 7.2 Hz, 3H, CH$_3$); 1.28 (m, 2H, CH$_2$); 2.03 (m, 1H, CH$_2$); 2.21 (m, 1H, CH$_2$); 4.05 (s, 1H, Fc); 4.13 (s, 5H, Fc); 4.20 (s, 1H, Fc); 4.24 (s, 2H, Fc); 4.99 (dd, J = 10.8 Hz, 3.5, 1H, CH); 7.47 (s, 1H, Im(C-2)); 7.73 (s, 1H, Im(C-5)). ^{13}C NMR (CDCl$_3$, δ, ppm): 13.55 (CH$_3$); 19.58 (CH$_2$); 37.74 (CH$_2$); 59.53 (CH); 66.25 (C$_5$H$_4$); 67.14 (C$_5$H$_4$); 68.50 (C$_5$H$_4$); 69.07 (C$_5$H$_5$); 69.14 (C$_5$H$_4$); 87.14 (C$_5$H$_4$-ipso); 117.83 (Im(C-2)); 135.20 (Im (C-5)); 147.93 (Im (C-NO$_2$)). Anal.: C 58.86; H 5.73; N 11.45. Calc. for C$_{17}$H$_{19}$FeN$_3$O$_2$: C, 58.87; H, 5.76; N, 11.44%.

2.2.5. 4-Nitro-1-(1-ferrocenyl-2-methylpropyl)-1H-imidazole (**4e**)

Yield 51%. Red-brown crystals, m. p. 138 °C. EI-MS, *m/z* (RI, %) 353 [M$^+$] (36). ^{1}H NMR (CDCl$_3$, δ, ppm): 0.80 (d, J = 6.7 Hz, 3H, CH$_3$); 0.90 (d, J = 6.7 Hz, 3H, CH$_3$); 2.15 (м, 1H, CH); 3.94 (s, 5H, Fc); 4.03 (s, 1H, Fc); 4.22 (s, 1H, Fc); 4.25 (s, 2H, Fc); 4.53 (d, J = 8.4 Hz, 1H, CH); 7.59 (s, 1H, Im(C-2)); 7.89 (s, 1H, Im(C-5)). ^{13}C NMR (CDCl$_3$, δ, ppm): 19.63 (CH$_3$); 20.51 (CH$_3$); 35.35 (CH); 65.65 (CH); 66.60 (C$_5$H$_4$);

67.67 (C_5H_4); 68.88 (C_5H_5); 69.19 (C_5H_4); 69.50 (C_5H_4); 86.88 (C_5H_4-*ipso*); 118.58 (Im(C-2)); 135.91 (Im (C-5)); 147.78 (Im (C-NO_2)). Anal.: C 58.84; H 5.74; N 11.46. Calc. for $C_{17}H_{19}FeN_3O_2$: C, 58.87; H, 5.76; N, 11.44%.

2.2.6. 4-Nitro-1-(ferrocenyl(phenyl)methyl)-1H-imidazole (4f)

Yield 47%. Yellow-brown crystals, m. p. 68°C. EI-MS, *m/z* (RI, %) 387 [M^+] (50). ^{1}H NMR (CDCl$_3$, δ, ppm): 4.04 (s, 1H, Fc); 4.06 (s, 5H, Fc); 4.21 (s, 1H, Fc); 4.32 (s, 1H, Fc); 4.36 (s, 1H, Fc); 6.22 (s, 1H, CH); 7.25 (m, 2H, Ph); 7.34 (s, 1H, Im(C-2)); 7.43 (m, 3H, Ph); 7.59 (s, 1H, Im (C-5)). ^{13}C NMR (CDCl$_3$, δ, ppm): 63.51 (CH); 68.23 (C_5H_4); 68.51 (C_5H_4); 69.29 (C_5H_5); 69.52 (C_5H_4); 69.54 (C_5H_4); 84.59 (C_5H_4-*ipso*); 119.16 (Im (C-5)); 127.41 (C_6H_5); 129.07 (C_6H_5); 129.12 (C_6H_5); 135.62 (C_6H_5); 138.15 (Im(C-2)); 147.60 (Im (C-NO_2)). Anal.: C 62.05; H 4.44; N 10.89. Calc. for $C_{20}H_{17}FeN_3O_2$: C, 62.04; H, 4.43; N, 10.85%.

2.2.7. 4-Nitro-1-((2-chlorophenyl)ferrocenylmethyl)-1H-imidazole (4g)

Yield 52%. Red-brown crystals, m. p. 149°C. EI-MS, *m/z* (RI, %) 421 [M^+] (36). ^{1}H NMR (CDCl$_3$, δ, ppm): 4.10 (s, 1H, Fc); 4.14 (s, 5H, Fc); 4.19 (s, 1H, Fc); 4.38 (s, 2H, Fc); 6.77 (s, 1H, CH); 7.17 (d, $J = 9.2$ Hz, 1H, Ph); 7.31 (m, 1H, Ph); 7.37 (m, 1H, Ph); 7.49 (s, 1H, Im(C-2)); 7.52 (m, 1H, Ph); 7.65 (s, 1H, Im(C-5)). ^{13}C NMR (CDCl$_3$, δ, ppm): 59.38 (CH); 67.53 (C_5H_4); 68.37 (C_5H_4); 69.04 (C_5H_4); 69.36 (C_5H_5); 69.75 (C_5H_4); 84.6 (C_5H_4-*ipso*); 118.98 (Im(C-5)); 127.62 (C_6H_4); 129.49 (C_6H_4); 130.29 (C_6H_4); 130.63 (C_6H_4); 133.46 (C_6H_4(C-*ipso*)); 135.91 (C_6H_4(C-Cl)); 135.98 (Im(C-2)); 147.60 (Im(C-NO_2)). Anal.: C 56.92; H 3.80; N 9.96. Calc. for $C_{20}H_{16}ClFeN_3O_2$: C, 56.97; H, 3.82; N, 9.97%.

2.2.8. 4-Nitro-1-((2-iodophenyl)ferrocenylmethyl)-1H-imidazole (4h)

Yield 52%. Yellow crystals, m. p. 85°C. EI-MS, *m/z* (RI, %) 513 [M^+] (45). ^{1}H NMR (CDCl$_3$, δ, ppm): 4.02 (s, 1H, Fc); 4.15 (s, 5H, Fc); 4.19 (s, 1H, Fc); 4.33 (s, 1H, Fc); 4.36 (s, 1H, Fc); 6.59 (s, 1H, CH); 7.11 (m, 2H, Ph); 7.36 (m, 1H, Ph); 7.52 (s, 1H, Im(C-2)); 7.62 (s, 1H, Im(C-5)); 7.93 (m, 1H, Ph). ^{13}C NMR (CDCl$_3$, δ, ppm): 67.02 (CH); 67.27 (C_5H_4); 67.95 (C_5H_4); 68.77 (C_5H_4); 69.43 (C_5H_5); 69.79 (C_5H_4); 85.47 (C_5H_4-*ipso*); 100.37 (C_6H_4 (C-I)); 119.05 (Im(C-5)); 129.06 (C_6H_4); 129.45 (C_6H_4); 131.04 (C_6H_4); 136.25 (Im(C-2)); 140.39 (C_6H_4); 140.51 (C_6H_4); 147.62 (Im(C-NO_2)). Anal.: C 46.76; H 3.20; N 8.19. Calc. for $C_{20}H_{16}FeIN_3O_2$: C, 46.82; H, 3.14; N, 8.19%.

2.2.9. 2-Methyl-4-nitro-1-(ferrocenylmethyl)-1H-imidazole (5a)

Yield 43%. Yellow-orange crystals, m. p. 89°C. EI-MS, *m/z* (RI, %) 325 [M^+] (71). ^{1}H NMR (CDCl$_3$, δ, ppm): 2.42 (s, 3H, CH$_3$); 4.18 (s, 5H, Fc); 4.20 (s, 2H, Fc); 4.24 (s, 2H, Fc); 4.79 (s, 2H, CH$_2$); 7.58 (s, 1H, Im(C-5)). ^{13}C NMR (CDCl$_3$, δ, ppm): 13.17 (CH$_3$); 46.96 (CH$_2$); 68.31 (C_5H_4); 68.77 (C_5H_4); 68.84 (C_5H_5); 69.33 (C_5H_4); 69.35 (C_5H_4); 79.88 (C_5H_4-*ipso*); 119.33 (Im(C-5)); 144.01 (Im(C-2)); 145.84 (Im(C-NO_2)). Anal.: C 54.80; H 4.72; N 12.78. Calc. for $C_{15}H_{15}FeN_3O_2$: C, 55.41; H, 4.65; N, 12.92%.

2.2.10. 2-Methyl-4-nitro-1-(1-ferrocenylethyl)-1H-imidazole (5b)

Yield 52%. Yellow-orange crystals, m. p. 83°C. EI-MS, *m/z* (RI, %) 339 [M^+] (87). ^{1}H NMR (CDCl$_3$, δ, ppm): 1,78 (d, $J = 6.9$ Hz, 3H, CH$_3$); 2.47 (s, 3H, CH$_3$); 4.08 (s, 1H, Fc); 4.17 (s, 5H, Fc); 4.21 (s, 1H, Fc); 4.25 (s, 2H, Fc); 5.15 (q, $J = 6.9$ Hz, 1H, CH); 7.84 (s, 1H, Im(C-5)). ^{13}C NMR (CDCl$_3$, δ, ppm): 13.31 (CH$_3$ (Im)); 21.18 (CH$_3$); 53.03 (CH); 65.84 (C_5H_4); 67.77 (C_5H_4); 68.53 (C_5H_4); 69.01 (C_5H_5); 69.35 (C_5H_4); 86.63 (C_5H_4-*ipso*); 117.51 (Im(C-5)); 143.52 (Im(C-2)); 146.10 (Im(C-NO_2)). IR (v, cm^{-1}): 3150, 3094, 2984, 1537 (s, NO_2), 1498 (s, NO_2), 1447, 1392 (s, NO_2), 1380 (s, NO_2), 1263, 1144, 1122, 1106, 829, 752, 508, 483. Anal.: C 58.69; H 5.79; Fe 15.32 N 11.37. Calc. for $C_{16}H_{17}FeN_3O_2$: C, 58.87; H, 5.76; N, 11.44%.

2.2.11. 2-Methyl-4-nitro-1-(1-ferrocenylpropyl)-1H-imidazole (5c)

Yield 54%. Orange powder, m. p. 141°C. EI-MS, *m/z* (RI, %) 353 [M^+] (57). ^{1}H NMR (CDCl$_3$, δ, ppm): 0.92 (t, $J = 7.6$ Hz, 3H, CH$_3$); 2.05 (m, 1H, CH$_2$); 2.37 (m, 1H, CH$_2$); 2.49 (s, 3H, CH$_3$); 4.05 (s, 1H, Fc); 4.16 (s, 5H, Fc); 4.19 (s, 1H, Fc); 4.21 (s, 1H, Fc); 4.23 (s, 1H, Fc); 4.86 (dd, $J = 10.9$ Hz, 3.6, 1H, CH); 7.62 (s, 1H, Im(C-5)). ^{13}C NMR (CDCl$_3$, δ, ppm): 11.09 (CH$_3$); 13.72, (CH$_3$ (Im)); 28.91 (CH$_2$); 59.26 (CH); 66.23 (C_5H_4); 67.07 (C_5H_4); 68.31 (C_5H_4); 69.04 (C_5H_5); 69.10 (C_5H_4); 87.46 (C_5H_4-*ipso*); 117.34 (Im (C-5)); 144.39 (Im (C-2)); 146.81 (Im (C- NO_2)). Anal.: C 58.85; 5 4.74; N 11.49. Calc. for $C_{17}H_{19}FeN_3O_2$: C, 58.87; H, 5.76; N, 11.44%.

2.2.12. 2-Methyl-4-nitro-1-(1-ferrocenylbutyl)-1H-imidazole (5d)

Yield 87%. Yellow crystals, m. p. 168°C. EI-MS, *m/z* (RI, %) 367 [M^+] (53). ^{1}H NMR (CDCl$_3$, δ, ppm): 0.98 (t, $J = 7.2$ Hz, 3H, CH$_3$); 1.25 (m, 2H, CH$_2$); 2.04 (m, 1H, CH$_2$); 2.25 (m, 1H, CH$_2$); 4.05 (s, 1H, Fc); 4.15 (s, 5H, Fc); 4.18 (s, 1H, Fc); 4.23 (s, 1H, Fc); 4.95 (dd, $J = 10.7$ Hz, 3.6, 1H, CH); 7.64 (s, 1H, Im(C-5)). ^{13}C NMR (CDCl$_3$, δ, ppm): 13.71 (CH$_3$ (Im)); 19.57 (CH$_3$); 37.75 (CH$_2$); 57.39 (CH$_2$); 66.25 (CH); 67.00 (C_5H_4); 68.28 (C_5H_4); 69.04 (C_5H_5); 87.67 (C_5H_4-*ipso*); 117.36 (Im(C-5)); 144.25 (Im(C-2)); 146.59 (Im(C-NO_2)). Anal.: C 58.92; H 5.71; N 11.47. Calc. for $C_{18}H_{21}FeN_3O_2$: C, 58.87; H, 5.76; N, 11.44%.

2.2.13. 2-Methyl-4-nitro-1-(1-ferrocenyl-2-methylpropyl)-1H-imidazole (5e)

Yield 47%. Yellow crystals, m. p. 124°C. EI-MS, *m/z* (RI, %) 367 [M^+] (38). ^{1}H NMR (CDCl$_3$, δ, ppm): 0.79 (d, $J = 6.4$ Hz, 3H, CH$_3$); 0.87 (d, $J = 6.8$ Hz, 3H, CH$_3$); 2.01 (m, 1H, CH); 2.59 (s, 3H, CH$_3$); 3.89 (s, 5H, Fc); 4.05 (s, 1H, Fc); 4.20 (s, 2H, Fc); 4.27 (s, 1H, Fc); 4.38 (d, $J = 9.6$ Hz, 1H, CH); 7.84 (s, 1H, Im(C-5)). ^{13}C NMR (CDCl$_3$, δ, ppm): 13.99 (CH$_3$ (Im)); 20.08 (CH$_3$); 20.42 (CH$_3$); 36.14 (CH); 64.13 (CH); 67.19 (C_5H_4); 68.69 (C_5H_5); 69.25 (C_5H_4); 69.91 (C_5H_4); 87.84 (C_5H_4-*ipso*); 117.36 (Im(C-5)); 144.60 (Im(C-2)); 153.14 (Im (C-NO_2)). Anal.: C 58.79; H 5.74; N 11.41. Calc. for $C_{18}H_{21}FeN_3O_2$: C, 58.87; H, 5.76; N, 11.44%.

2.2.14. 2-Methyl-4-nitro-1-(ferrocenyl(phenyl)methyl)-1H-imidazole (5f)

Yield 55%. Yellow crystals, m. p. 132°C. EI-MS, *m/z* (RI, %) 401 [M^+] (72). ^{1}H NMR (CDCl$_3$, δ, ppm): 2.24 (s, 3H, CH$_3$); 3.87 (s, 1H, Fc); 4.08 (s, 5H, Fc); 4.23 (s, 1H, Fc); 4.30 (s, 1H, Fc); 4.38 (s, 1H, Fc); 6.14 (s, 1H, CH); 7.22 (m, 2H, Ph); 7.39 (m, 1H, Ph); 7.41 (m, 2H, Ph); 7.45 (s, 1H, Im(C-5)). ^{13}C NMR (CDCl$_3$, δ, ppm): 13.63 (CH$_3$); 62.04 (CH); 68.49 (C_5H_4); 68.56 (C_5H_4); 69.10 (C_5H_4); 69.18 (C_5H_5); 69.93 (C_5H_4); 84.84 (C_5H_4-*ipso*); 119.15 (Im(C-5)); 127.07 (C_6H_5); 128.59 (C_6H_5); 128.78 (C_6H_5); 137.17 (C_6H_5); 144.45 (Im(C-2)); 145.47 (Im (C-NO_2)). IR (v, cm^{-1}): 3168, 3096, 3030, 2923, 1532 (s, NO_2), 1487 (s, NO_2), 1455, 1380 (s, NO_2), 1263, 1151, 1107, 1003, 993, 820, 750, 730, 703, 506, 488, 479. Anal.: C 62.85; H 4.70; N 10.52. Calc. for $C_{21}H_{19}FeN_3O_2$: C, 62.86; H, 4.77; N, 10.47%.

2.2.15. 2-Methyl-4-nitro-1-((2-chlorophenyl)ferrocenylmethyl)-1H-imidazole (5g)

Yield 55%. Orange crystals, m. p. 92°C. EI-MS, *m/z* (RI, %) 435 [M^+] (63). ^{1}H NMR (CDCl$_3$, δ, ppm): 2.34 (s, 3H, CH$_3$); 4.03 (s, 1H, Fc); 4.12 (s, 5H, Fc); 4.19 (s, 1H, Fc); 4.36 (s, 1H, Fc); 4.37 (s, 1H, Fc); 6.61 (s, 1H, CH); 7.12 (m, 1H, Ph); 7.29−7.38 (m, 2H, Ph); 7.47 (s, 1H, Im(C-5)); 7.52 (m, 1H, Ph). ^{13}C NMR (CDCl$_3$, δ, ppm): 13.81 (CH$_3$ (Im)); 57.46 (CH); 67.51 (C_5H_4); 68.69 (C_5H_4); 69.39 (C_5H_5); 69.88 (C_5H_4); 84.19 (C_5H_4-*ipso*); 119.18 (Im(C-5)); 127.73 (C_6H_4); 129.08 (C_6H_4); 130.14 (C_6H_4); 132.86 (C_6H_4 (C-*ipso*)); 135.41 (C_6H_4 (C-Cl)); 144.60 (Im(C-2)); 145.63 (Im(C-NO_2)). Anal.: C 57.79; H 4.14; N 9.69. Calc. for $C_{21}H_{18}ClFeN_3O_2$: C, 57.89; H, 4.16; N, 9.64%.

2.2.16. 2-Methyl-4-nitro-1-((2-iodophenyl)ferrocenylmethyl)-1H-imidazole (**5h**)

Yield 43%. Orange crystals, m. p. 84°C. EI-MS, m/z (RI, %) 527 [M$^+$] (83). ^{1}H NMR (CDCl$_3$, δ, ppm): 2.40 (s, 3H, CH$_3$); 4.09 (s, 2H, Fc); 4.15 (s, 5H, Fc); 4.38 (s, 2H, Fc); 6.39 (s, 1H, CH); 7.08 (m, 1H, Ph); 7.37–7.44 (m, 2H, Ph); 7.56 (s, 1H, Im(C-5)); 7.98 (m, 1H, Ph). ^{13}C NMR (CDCl$_3$, δ, ppm): 14.87 (CH$_3$); 65.55 (CH); 67.12 (C$_5$H$_4$); 68.95 (C$_5$H$_4$); 69.29 (C$_5$H$_4$); 69.52 (C$_5$H$_4$); 69.59 (C$_5$H$_5$); 85.03 (C$_5$H$_4$-ipso); 100.12 (C$_6$H$_4$(C-I)); 119.39 (Im(C-5)); 128.79(C$_6$H$_4$); 129.13 (C$_6$H$_4$); 130.64 (C$_6$H$_4$); 140.02 (C$_6$H$_4$ (C- ipso)); 140.35 (C$_6$H$_4$); 145.12 (Im(C-2)); 146.57 (Im(C-NO$_2$). Anal.: C 47.81; H 3.45; N 7.99. Calc. for C$_{21}$H$_{18}$FeIN$_3$O$_2$: C, 47.85; H, 3.44; N, 7.97%.

2.3. X-ray crystallography

Single crystals of C$_{21}$H$_{19}$FeN$_3$O$_2$ (**5f**) were orange prisms crystallized from acetone. A suitable crystal was selected from crud product, and intensities of reflections were measured on a Bruker SMART CCD 1K diffractometer in Centre for molecular composition studies of INEOS RAS. The crystal was kept at room temperature during data collection. The structure was solved with the XS structure solution program [42] using Direct Methods and refined with the ShelXL [43] refinement package using Least Squares minimisation. Molecular graphics were drawn using OLEX2 program [44]. All crystallographic data were submitted to Cambridge Crystallographic Data Centre (CCDC deposition number 1816071). Selected crystallographic parameters were summarized in Table 1.

Table 1
Crystallographic data for **5f**.

Empirical formula	C$_{21}$H$_{19}$FeN$_3$O$_2$
Formula weight	401.24
T, K	293
Space group, Z	P2$_1$/n, 4
a, Å	12.6743 (7)
b, Å	10.2407 (6)
c, Å	14.6595 (9)
α, °	90
β, °	98.4280 (10)
γ, °	90
V, Å^3	1882.16 (19)
Density$_{calc}$, g·cm^{-3}	1.416
μ, cm^{-1}	8.22
F (000)	832
2θ$_{max}$, °	57.99
Reflections collected	14116
Independent reflections	4972
Reflections with I > 2σ(I)	3690
Parameters	245
R1 [I > 2σ(I)]	0.0387
wR2 [all reflections]	0.1062
GOF	1.028
Residual electron density, e·Å^{-3} (ρ$_{min}$/ρ$_{max}$)	0.544/-0.215

2.4. Racemic resolution by HPLC

The following chiral columns (250 × 4.6 mm, 5 μm) were used: 3-AmyCoat, Chiracel OD, Chiracel OJ. Chromatographic resolution was carried out on an HPLC system (Advanced Separation
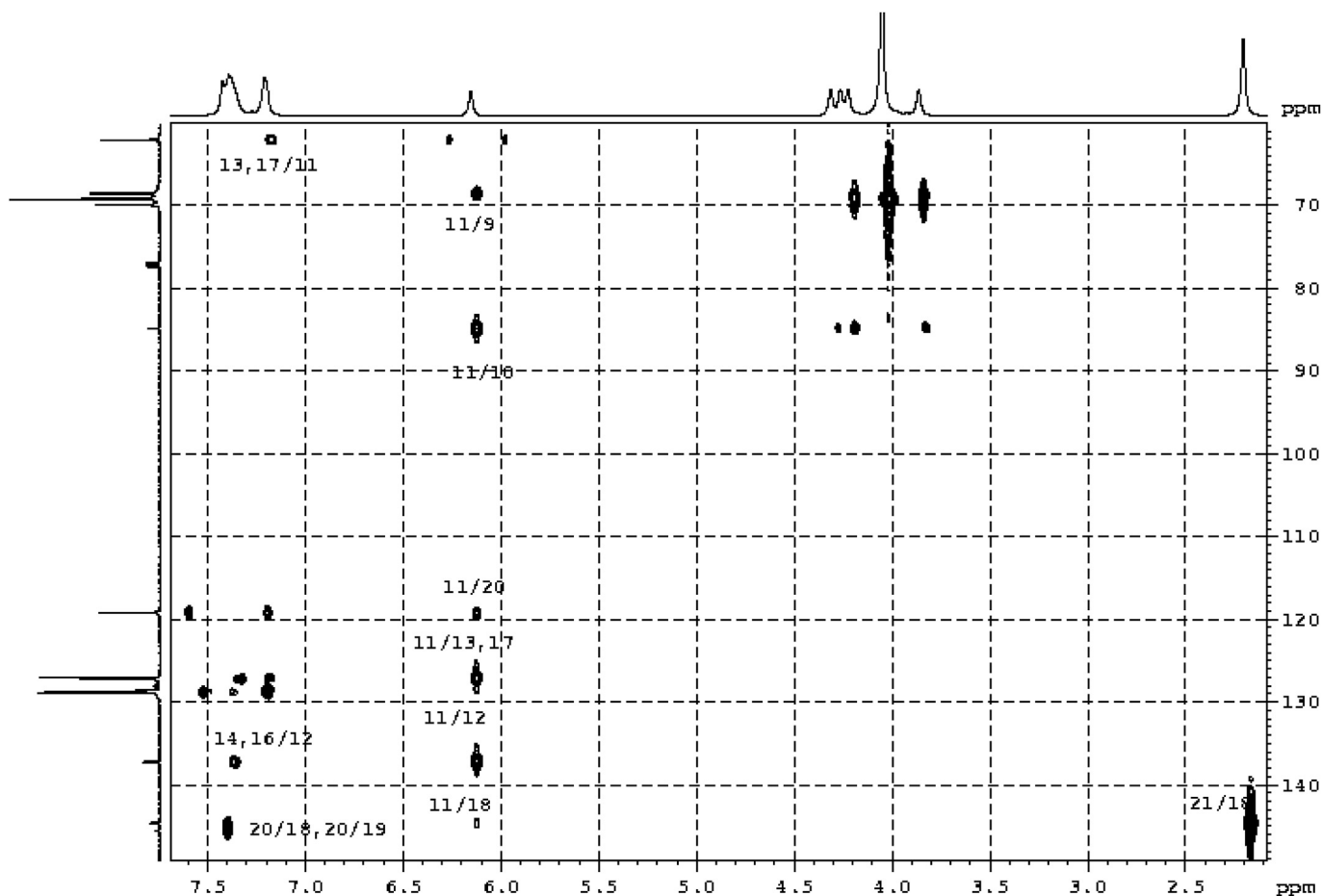

Fig. 1. . Fragment of HMBC spectrum of 1N-(ferrocenylbenzyl)-2-methyl-4-nitro–imidazole (**5f**).

Thechnologies, Inc., Whippany, NJ, USA), with a Bruker LC 31 instrument equipped with a UV detector (254 nm); the flow rate was 1.0 mL min^{-1} at an ambient temperature.

2.5. Cytotoxicity

Rat hepatoma HTC cells were cultured in the DMEM/F12 medium with addition of 10% fetal calf serum (HyClone, USA), *L*-glutamine (PanEco, Russia) and gentamicin as antibiotic (PanEco, Russia). The cells were seated on 96-well plates in concentration of 2×10^5 per well and cultured in a CO_2 incubator at 37 °C for 24 h. Then the solutions of 1*N*-(ferrocenylethyl)-4-nitro-imidazole (**4b**), 1*N*-(ferrocenylbutyl)-4-nitro-imidazole (**4d**), 1*N*-(ferrocenylethyl)-2-methyl-4-nitroimidazole (**5b**) and 1*N*-(ferrocenylpropyl)-2-methyl-4-nitroimiazole (**5c**) in increasing concentrations of 5.0, 10.0, 15.0, 20.0, and 25.0 μM were added to the cells. As a control was the solution of 0.68 M DMSO in water. After 2, 24 and 48 h the viability was assessed by the MTT assay. All experiments were performed in triplicate at each concentration level and repeated three times. The data have been given in terms of percent growth inhibition relative to untreated controls (see Supplementary Materials).

2.6. Antitumor test

Carcinoma 755 (Ca755) was transplanted subcutaneously to the inbred mice BDF$_1$/f1(C57Bl6 × DBA2)/males, a hybrid line of C57Bl6 females and DBA2 males, with weight 18−20 g, in accordance with the standard procedure. The agents **4b** and 1*N*-FcCH(CH$_3$)-Im were administered on the next day after tumor inoculation. The tested daily doses were 10.0 and 20.0 mg kg^{-1} (total doses were 50.0 and 100.0 mg kg^{-1}). Ethanol-water solutions (10% by volume) of compounds **4b** and FcCH(CH$_3$)-Im were administered intraperitoneally in daily doses (Table 3) five times every day starting from the next day after tumor inoculation. Each group comprised five to seven animals, including the control group of animals.

The kinetics of tumor growth was studied by measurement of tumor size during the whole period of tumor development. Two cross-coupling tumor sizes were measured and the volume of the tumor was calculated as $V = ab^2/2$, where a is the length and b is the width and the height of the tumor. As estimated previously, the density of tumor tissue is equal to 1.0 g cm^{-3}. So it is assumed that the weight of tumor in grams is equal to the volume of tumor in cm^3. The index of tumor growth inhibition (TGI) was calculated as

(C − T)/C, %, where C and T are the mean tumor weight in groups of control and treated animals, respectively.
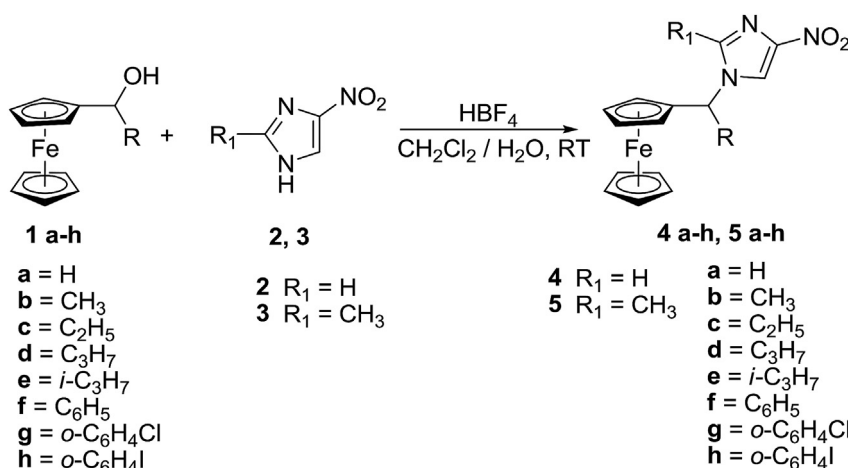
3. Results and discussion

3.1. Synthesis

Imidazoles and benzimidazoles, being the central ingredients in many drugs, are often used for chemical modifications by ferrocene for medicinal investigations [12,16,45,46].

A high effective approach to ferrocenyalkylated imidazole and benzimidazoles was realized previously in neutral media using *N,N'*-carbonyldiimidazole or *N,N'*-thiobenzimidazoles as nucleophilic agents [47,48]. Imidazole has the highest basicity among five-membered nitrogen-containing heterocycles in living organisms with basic pK_a 6.98. Nitro-imidazoles have significantly lower basicity than imidazole due to electron-withdrawing effect of the nitro-group. So, we carried out the Fc-alkylation reaction of nitro-imidazoles through *in situ* generation of thermodynamically stable ferrocenylcarbenium ions FcC$^+$H(R) by adding of strong fluoroboric acid and equimolar ratio of reagents avoiding protonation of heterocycles. An advantage of this method is the simplicity, the accessibility of initial reagents and the possibility to vary the lipophility of final products by changing ferrocenyl alcohols. Ferrocenylalkyl nitro-imidazoles (**4a–f, 5 a–f**, Scheme 1) were synthesized *via* the reaction of 4-nitro-benzimidazole (**2**) or 2-methyl-4-nitro-benzimidazole (**3**) with eight different ferrocenyl alcohols, FcCHR (OH) (**1a-h**), in methylene dichloride at room temperature in the presence of 45% aqueous fluoroboric acid (with an equimolar ratio of ferrocenyl alcohol, nitro-imidazole and the acid). The products of the reactions were isolated in satisfactory to good yields (43−87%) without column chromatography. To prevent the oxidation of the final product during the work-up, ascorbic acid was added. Fc-Alkylation appeared to be a regiospecific process and 1-*N*-ferrocenylalkyl nitro-imidazoles were the only products formed, which were proved basing on NMR spectral data.

The ^{1}H NMR spectra of compounds **4a–f** and **5 a–f**, in CDCl$_3$ shows several sets of signals assigned, respectively, to the protons of the substituted and unsubstituted cyclopentadienyl rings, to the CH(R)-bridge protons, their substituents R, and to the protons of nitro-imidazoles and their methyl substituent. The assignments of the signals of the ^{13}C NMR spectra of Fc-compounds with 4-nitro-imidazole and 2-methyl-4-nitro-imidazole were based on HSQC spectra. The structures of compounds were assigned on the basis of

1 a-h

a = H
b = CH$_3$
c = C$_2$H$_5$
d = C$_3$H$_7$
e = *i*-C$_3$H$_7$
f = C$_6$H$_5$
g = *o*-C$_6$H$_4$Cl
h = *o*-C$_6$H$_4$I

2, 3

2 R$_1$ = H
3 R$_1$ = CH$_3$

4 a-h, 5 a-h

4 R$_1$ = H
5 R$_1$ = CH$_3$

a = H
b = CH$_3$
c = C$_2$H$_5$
d = C$_3$H$_7$
e = *i*-C$_3$H$_7$
f = C$_6$H$_5$
g = *o*-C$_6$H$_4$Cl
h = *o*-C$_6$H$_4$I

Scheme 1. Synthesis of ferrocenylalkyl-4-nitro-imidazoles (**4a–h**, **5a–h**) from ferrocenyl alcohols (**1a–h**) and 4-nitro-imidazoles (**2, 3**).
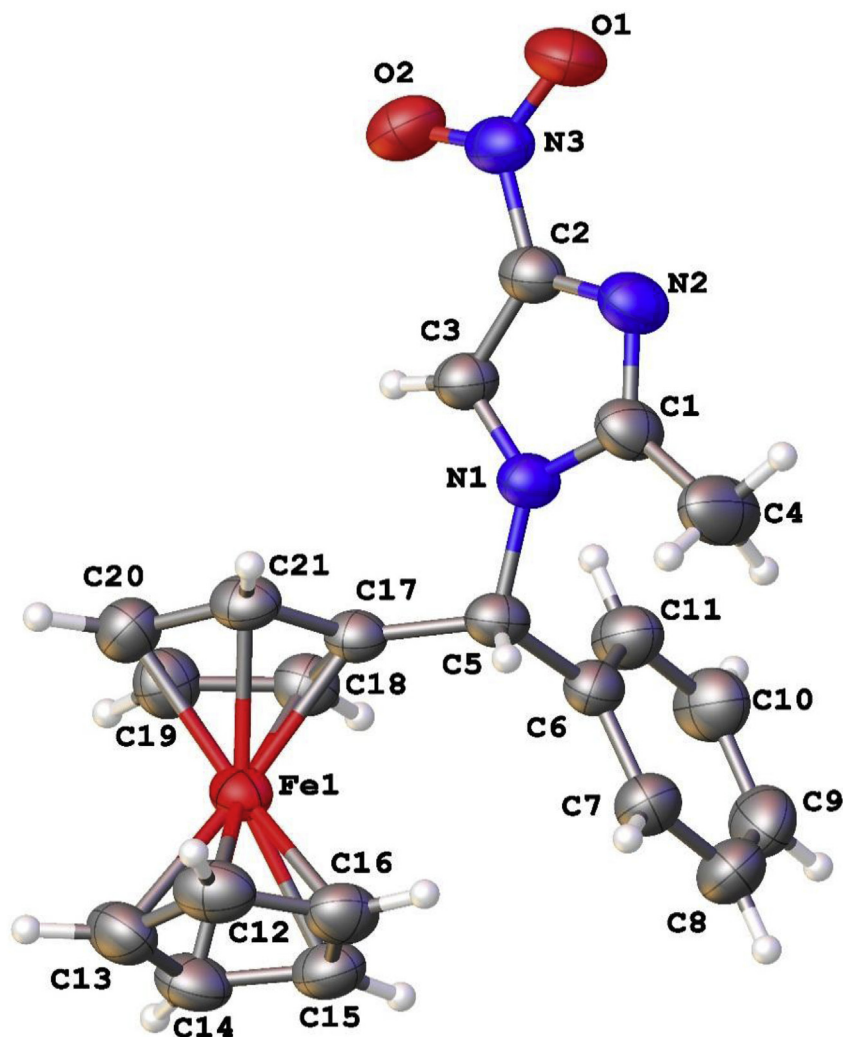
Fig. 2. Molecular structure of **5f** presented in thermal ellipsoids at 50% probability. Selected lengths, (Å) and angles (°), C5-C17 1.508 (3); N1-C5 1.486 (2); C5-C6 1.519 (3); Fe1-C17 2.0310 (17); N1-C1 1.312 (3); N1-C3 1.354 (2); N3-C2 1.356 (3); N1-C5-C17 109.55 (14); N1-C5-C6 111.04 (15); C5-C17-C21 124.45 (16); C6-C5-C17 113.46 (15).

^{1}H and ^{13}C NMR spectra and ^{1}H/^{13}C heteronuclear correlations. Particularly in the HMBC spectrum of 1*N*-(ferrocenylbenzyl)-2-methyl-4-nitro-imidazole (**5f**) (Fig. 1), there are essential correlations between singlet at 6.14 ppm attributed to the CH linked to the ferrocene moiety (on Fig. 1 C-11), and unsubstituted C-atom in imidazole (C-20, 119.15 ppm) and carbon atom at Me-group (C-18, 144.45 ppm) of 2-methyl-4-nitro-imidazole. There are no correlations between CH-linked proton and carbon atom bearing NO$_2$-group imidazole (C-19). Thus, ferrocenylalkylation proceeded in 1*N*-position of nitro-imidazole ring and appeared to be regiospecific process. Regiospecificity of alkylation of the others derivatives was proved in the same way.

3.2. Crystal structure

According to single crystal X-ray diffraction study the bond lengths in ferrocene and imidazole moieties are very close to those in previously studied derivatives of α-(hydroxy)alkyl ferrocenes (see caption to Fig. 2and X-ray data for Fc-imidazoles [12]). Moreover, the nitro group in the heterocycle does not cause any change in the bond length N1-C5 connecting imidazole and α-carbon atom. For **5f** this length is equaled 1.486 (2) Å, for Fc-CH(Ph)Im — 1.487 Å [12]. The most noticeable intermolecular interactions in the crystal packing of **5f** are weak the C-H … π interactions between ferrocene and imidazole fragments. Nitro groups participated in weak C-H…O bonds with ferrocene groups and methyl groups.

3.3. Chiral resolutions by HPLC

In this section, racemic ferrocenylalkyl nitro-imidazoles were considered. Chiral centers in the investigated compounds (**4b**–**h** and **5b**–**h**) are represented by the carbon atom connecting a ferrocene moiety with a heterocycle. These racemic mixtures were successfully separated into enantiomers on silica packings modified by amylose as chiral selector, using the HPLC analytical method.

Earlier, this method of separation was initially applied for racemic ferrocene compounds carrying various simple substituents [49]. The chiral sorbents based upon β- and γ-cyclodextrins turned out to be effective in this case, as well as for the separation of ferrocene pyrazoles [36]. To separate mixtures of racemic ferrocene derivatives having bulky substituents such as ferrocenylalkyl azoles [35,48] or ferrocenylalkyl thiopyrimidines [32], modified cellulose was used as the chiral stationary phase. Columns with amylose derivatives were used to separate into enantiomers ferrocene derivatives of mercaptoazoles [50].

The enantiomeric resolution analytical data are summarized in

Table 2
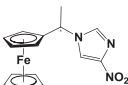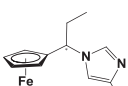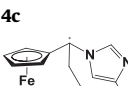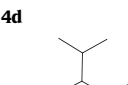Enantiomeric resolution of **4b-h** and **5b-h** racemic mixtures on column 3-Amy Coat.
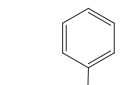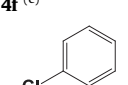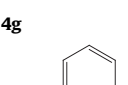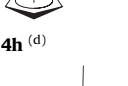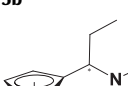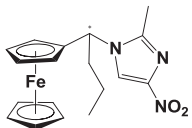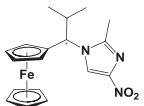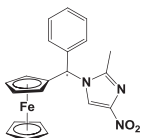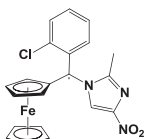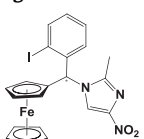
Molecule[a] and compound number	HPLC data[b]		
	Retention factor, k'_1	Retention factor, k'_2	Separation factor, α
4b	10.47	13.52	1.29
4c	9.32	11.17	1.20
4d	6.16	10.13	**1.64**
4e	5.20	7.35	1.41
4f [c]	11.41	13.28	1.16
4g	7.15	8.03	1.12
4h [d]	48.93	61.03	1.25
5b	4.90	6.34	1.29
5c	4.77	5.63	1.18

Table 2 (continued)

Molecule[a] and compound number	HPLC data[b]		
	Retention factor, k'_1	Retention factor, k'_2	Separation factor, α
5d	3.73	4.07	1.09
5e	5.59	10.46	1.87
5f	3.33	4.93	1.48
5g	4.27	8.86	**2.07**
5h	5.04	9.52	**1.89**

[a] * in the structures means the stereogenic centre; [b] Mobile phase, hexane—isopropanol 9:1 (v/v); [c] For compound **4f**, experimental data were obtained on the modified cellulose, column Chiralcel OD; [d] For compound **4h**, experimental data were obtained on the modified cellulose, column Chiralcel OJ.

Table 3
Antitumor activity of 1N-(ferrocenylethyl)-4-nitroimidazole (4b) against Ca755 carcinoma *in vivo*.

Compound	Daily dose, mg kg^{-1}	Adenocarcinoma 755	
		Mean tumor weight, g	Tumor growth inhibition, % [a]
FcCH(CH$_3$)Im[b]	10.0	4.5 ± 0.7	+50 (stimulation)
FcCH(CH$_3$)Im	20.0	4.8 ± 0.7	+60 (stimulation)
Control	—	3.0 ± 0.6	—
4-NO$_2$FcCH(CH$_3$)Im (**4b**)	10.0	3.2 ± 0.4	22
4-NO$_2$FcCH(CH$_3$)Im (**4b**)	20.0	6.0 ± 0.7	+46 (stimulation)
Control	—	4.1 ± 0.6	—

Initial solvent, physiological solution-ethanol 90:10, percentage by volume; drug administration, intraperitoneal; during five days after tumor inoculation.
[a] Evaluation of the index of tumor growth inhibition (%), day 14 after tumor Ca755 inoculation for FcCH(CH$_3$)Im; day 16 after tumor Ca755 inoculation for 4b.
[b] This compound was prepared according Reference [47].

Table 2. We successfully separated all 14 pairs of investigated compounds. Some interesting conformities should be noted. First, retention factors k'_1 and k'_2 for compounds **4b-4d** and **5b-5d** with aliphatic substituents R decrease with the growing chain length of R. For example, for **4b** and **4d** from k'_1 10.47 and k'_2 13.52 to 6.16 and 10.31, respectively; the same for derivatives with methyl group in the imidazole ring — from 4.90 to 6.34 for **5b**, and 3.73 and 4.07 for **5d**. It should be marked that methyl group in the heterocycle makes retention factors low. On the other hand, for phenyl containing compounds, vice versa, retention factors increase monotonously on introduction of chlorine and iodine substituents into the phenyl ring. For example, for **5f** k'_1 equals 3.33 and k'_2 equals 4.93; for **5h** 5.04 and 9.52, correspondingly. The most efficient HPLC separation was achieved in the case of compounds **5j** and **5h**, with halogen-containing phenyl linkers, $\alpha = 2.07$ for **5j** and $\alpha = 1.89$ for **5h** (Table 2).

The recognition mechanism on amylose is apparently connected, with the formation of specific hydrogen bonds between the strongly basic nitrogen atom of the corresponding heterocyclic fragments or/and nitro-groups and carbamate units of the modified

amylose.

3.4. Biological tests. cytotoxicity and antitumor activity

Since the end of the 1990s, increased interest in the biochemistry of ferrocenes has emerged, especially in the therapeutic areas of oncology [1,51]. At present, several significant review papers devoted to antitumor activities of ferrocene compounds including ferrocene-modified nucleic bases, nitrogen heterocycles, aromatic substances, and drugs were published [3–6,9,10,15]. This problem was carefully treated including mechanistic aspects [4,10,13]. According to recently published analysis, the most probable mechanism of the anticancer action of ferrocene compounds is the initiation of tumor cell apoptosis by protecting a telomere from the effect of telomerase and/or decreasing the telomerase activity [4]. Most likely, the development of therapeutic schemes for potential ferrocene-based drugs will continue in the near future.

Herein, in sequel of our works [12,35,50,52] ferrocene-modified nitro-imidazoles were investigated. Nitro-imidazole-based drugs are widely used in clinical and medicinal practices as anaerobic antimicrobial agents, namely, tinidazole, metronidazole, dimetridazole, ornidazole, nimorazole, secnidazole, satranidazol [53–55].

The exceptional role of imidazole derivatives in living systems is well known. They take part in the essential biochemical processes, since the imidazole ring is an indispensable structural fragment of nucleic acids, histidine and its decarboxylation product histamine.

At the same time, introduction of the ferrocene moiety in a biomolecule may change the normal route of their interactions with biological targets, pharmacokinetics and pharmacodynamics or the direction of binding [4,15]. Therefore, such approach in construction of new biologically active ferrocene-modified molecules is in progress now [29,56–59]. However, in contrast to the rather well developed ferrocene-based area, only limited representatives of ferrocene-containing nitro-imidazoles are known [46]. Thus, the synthesis of new ferrocene-based nitro-imidazoles as potential drug candidates is of current interest.

3.4.1. MTT assay. viability of the tested compounds

The viability of four ferrocene compounds, namely, 1N-(ferrocenylethyl)-4-nitro-imidazole (4b), 1N-(ferrocenylbutyl)-4-nitro-imidazole (4d), 1N-(ferrocenylethyl)-2-methyl-4-nitroimidazole (5b) and 1N-(ferrocenylpropyl)-2-methyl-4-nitroimiazole (5c) was evaluated by the MTT assay in increasing concentrations of 5.0, 10.0, 15.0, 20.0, and 25.0 μM. Rat hepatoma cell line (HTC) was used to study the sensitivity to ferrocene nitro-imidazoles with different alkyl substituents R (Scheme 1, from methyl for 4b and 5b to ethyl for 5c and propyl for 4d).

Cytotoxicity (%) = As/Amc × 100%, where As – optical density of tested sample, Amc – mean optical density of the control sample.

As shown in Table 4 (Supplementary materials), a distinct proliferative effect was observed for compound 4b during the incubation of the rat hepatoma cells for 2 h. However, this effect was not observed for incubation times approaching 24 h. Moreover, when the solution with a concentration of 25 μM was used, the cell viability decreased. Also it is noteworthy that the viability of rat hepatoma cells decreased with the increasing of the incubation time, when compound 4b was used in concentrations of 20 μM and 25 μM.

Compound 4d in a concentration of 5 μM leads to a decrease in the viability of cells by 17.6% in the rat hepatoma cell line HTC after

a 2-h cultivation. Increasing in the duration of culturing to 24 h is accompanied by a decrease of the cell viability, when this compound 4d is added in an amount of 10–25 μM. A 48-h incubation was not accompanied by a cytotoxic effect.

Compound 5b did not have any cytotoxic effect on the cells during the HTC culture incubation for 2, 24 and 48 h at the investigated concentrations. In contrast, some stimulation of the cell proliferation was observed during incubation with the compound in a concentration of 5 μM for 48 h.

Incubation of the compound 5c with the rat hepatoma cells was not accompanied by cytotoxic effects at the concentrations and time periods investigated. It should be noted that the increasing of proliferation activity of the cells during the incubating of the compounds with the HTC cells for 2 and 48 h and concentration of 25 μM.

As a result, no cytotoxic effects of 1N-(ferrocenyl)nitro-imidazoles 5b and 5c on the culture of rat hepatoma were found with the exception of compounds 4b and 4d at relatively high concentrations and long incubation periods (a day or more). This opens perspectives for further detailed study of the specific pharmacological properties of the compounds obtained.

3.4.2. Antitumor activity

The antitumor effects of ferrocene-based thymine [31], benzimidazole [12], thiopyrimidine [32], and thiobenzimidazole [50] against some solid tumor models such as Ca755 carcinoma (Ca755) and Lewis lung carcinoma (LLC) transplanted in mice have been studied earlier. The index of tumor growth inhibition as high as 95%, in comparison with control was achieved in some cases [32]. This effectiveness was comparable with that of cisplatin. It was marked that solid tumor models, namely, Ca755 carcinoma and Lewis lung carcinoma were considerably more sensitive to ferrocene compounds than ascite ones, such as L1210 and P388 leukemia.

For the assessment of antitumor activity Ca755 carcinoma was used. The tested doses were 10.0 and 20.0 mg kg^{-1}. The solutions were administered intraperitoneally. Tumor sizes were measured during the whole period of tumor growth. The index of tumor growth inhibition was calculated at the time point where the antitumor activity of the drug was maximal. This was after 16 days for 4b and after 12 days for its analog without nitro-group, FcCH(CH$_3$)Im. The results of activity against the above-mentioned murine tumor are summarized in Table 3. As seen from Table 3, carcinoma 755 is sensitive to both compounds. An insignificant antitumor effect of compound 4b was shown on Ca755 at the dose of 10.0 mg kg^{-1}. Moreover, 4b and FcCH(CH$_3$)Im, as was proved experimentally, stimulated the growth of solid tumor Ca755. Such an activity reversal phenomenon has not been observed before. This tumor model showed that some imidazole-based organometallics can exhibit stimulation effects.

To understand these unexpected facts we analyzed some biological results. Earlier, on solid tumor models an inverse dose–effect response was found for ferrocenylmethyl benzimidazoles [12] (Lewis lung carcinoma, dose 5.0 mg kg^{-1}, tumor growth inhibition 70%), ortho-carboxybenzoyl ferrocene sodium salt [60] (Ca755, dose 2.5 mg kg^{-1}, tumor growth inhibition 70%), 1N-ferrocenylmethyl thymine against Ca755, dose 2.5 mg kg^{-1}, tumor growth inhibition 70% [31], as well as for some other ferrocene derivatives [11], that is, a decrease of the dose gave an increase of the effects. Then, it was suggested that the found dose–efficiency dependence – the achievement of the maximum antitumor effect after application of the agent in a rather low doses – is typical for ferrocene compounds of this kind [31]. A preliminary conclusion

was being drawn: the anomalies in dose−effect response may be connected to the increased immunogenicity of the ferrocene derivatives. A large dose causes enhanced immune response and, as a consequence, the earlier destruction of the compound [31].

4. Conclusion

A series of nontoxic ferrocene nitro-imidazoles have been easily obtained in good yields by reaction of commercially or synthetically available ferrocene alcohols with nitro-imidazoles. X-ray structural data for racemic (R,S)-1-N-(benzyl ferrocenyl)-2-methyl-4-nitroimidazole (**5f**) were determined. The resulting enantiomers were resolved into enantiomers by analytical HPLC method. Cytotoxicity studies of (R,S)-ferrocenyl (alkyl) nitro-imidazoles were realized. Antitumor activity tests were made for compound **4b**.

DFT calculations and investigations into the unusual reactivity and biological properties of these novel ferrocene derivatives are in progress now in our laboratories.

Acknowledgments

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jorganchem.2018.06.019.

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