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Synthesis and biological evaluation of novel ethyl 2-amino-6-ferrocenyl-1,6-dihydropyrimidine-5-carboxylates and ethyl 2-amino-6-ferrocenylpyrimidine-5-carboxylates

Elena I. Klimova [a,*], Jessica J. Sanchez García [a], Tatiana Klimova [a], Teresa Ramírez Apan [b], Eduardo A. Vázquez López [a], Marcos Flores-Alamo [a], Marcos Martínez García [b]

[a] Facultad de Química, Universidad Nacional Autónoma de México, Cd. Universitaria, Coyoacán, C.P. 04510, México D.F., Mexico
[b] Universidad Nacional Autónoma de México, Instituto de Química, Cd. Universitaria, Coyoacán, C.P. 04510, México D.F., Mexico

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ABSTRACT

Reactions of ethyl 2-acyl-3-ferrocenylacrylates (acyl = acetyl, benzoyl, *p*-nitrobenzoyl) with guanidine and 1,1-dimethylguanidine furnish ethyl 2-amino-6-ferrocenyl-4-methyl(aryl)-1,6-dihydropyrimidine-5-carboxylates. Their oxidative dehydrogenation with $PhI(OAc)_2$ results in the corresponding ethyl 2-amino-6-ferrocenyl-4-methyl(aryl)pyrimidine-5-carboxylates. The structures of the synthesized compounds were established on the basis of the data from ^{1}H and ^{13}C NMR spectroscopy and confirmed by X-ray diffraction analysis. All compounds were tested *in vitro* against six human tumor cell lines U-251, PC-3,K-562, HCT-15, MCF-7 and SKLU-1 to assess their *in vitro* antitumor activity. The results suggest biological specificity towards PC-3 and K-562 cells for compounds **4a**–**c** at doses 50 μM, which are lower than *cis*-platin IC_{50}s in the two cell lines. Additionally, peritoneal mouse macrophages (M∅) were also evaluated for compounds **4a**–**f** and **5a**–**f**.

1. Introduction

Pyrimidines are widespread among natural bases [1], they are abundant constituents of medicines possessing pronounced antiviral [2], gastric antisecretory [3], diuretic [4], antimalarial [5], anti-HIV-1 [6,7] activities, *etc*. It is also known that in recent years, bioorganometallic chemistry has developed as a rapidly growing and maturing area which links classical organometallic chemistry to biology, medicine, and molecular biotechnology [8]. The properties of ferrocene and its derivatives attract steady interest of researchers in many fields of organic and organometallic chemistry. Wide range of studies of applied properties of ferrocene is due to its specific physical and chemical properties: high thermal stability, high vapor pressure, low toxicity, good solubility in organic solvents, and diverse chemical transformations [9]. The incorporation of ferrocenyl substituents into molecules of organic compounds frequently brings about the enhancement of the biological activity of the original compounds [10]. Many ferrocenyl-substituted nitrogen heterocycles, such as quinuclidine, triazole, benzimidazole, benzindazole, pyrazoline, pyrazole, pyrimidone, tetrahydropyridazine derivatives, *etc*., were reported [10c–19] to pertain to biologically active compounds. Therefore, the interest in the synthesis of novel ferrocenylpyrimidines is quite justified.

The starting compounds for the synthesis in the pyrimidine series usually bear two amino or an amino and an imino groups at the same carbon atom [10c] (urea, guanidine, amidine derivatives), which react with β-dicarbonyl compounds, ethyl cyanoacetate derivatives, α,β-enones, *etc*. The first ferrocenyl-substituted tetrahydropyrimidines of the type **1** were prepared by the coupling of thiourea [20–22] and urea [23] with ferrocenyl-α,β-enones under sonication or in the presence of bases (Scheme 1).

In the last years, new publicationes about the synthesis of ferrocenylpyrimidines from α,β-ferrocenylenones and derivatives of guanidines and amidines have appeared [24–26]. However, the approaches to the synthesis of ferrocenylpyrimidines and their properties remain largely unexplored so far.

In the present work, we accomplished the synthesis of functionalized ferrocenyldihydropyrimidines and ferrocenylpyrimidines by the coupling of guanidine and 1,1-dimethylguanidine with ethyl 2-acyl-3-ferrocenylacrylates. The use of 2-acylacrylates for the preparation of pyrimidine derivatives has not hitherto been documented.

* Corresponding author. Tel./fax: +52 555622 5371.
E-mail address: eiklimova@yahoo.com.mx (E.I. Klimova).

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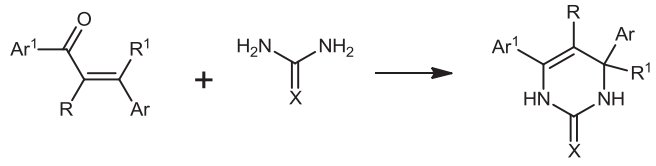


Scheme 1.

2. Results and discussion

2.1. Chemistry

Ethyl (*E*)- and (*Z*)-2-acetyl- (**2a**, **2b**), (*E*)-2-benzoyl- (**2c**), and (*E*)-2-(*p*-nitrobenzoyl)-3-ferrocenylacrylates (**2d**) served as the starting compounds (Fig. 1).

They were prepared by coupling ferrocenecarbaldehyde with ethyl acetoacetate, ethyl benzoylacetate, and ethyl (*p*-nitrobenzoyl) acetate, respectively, in the presence of piperidinium acetate [27,28].

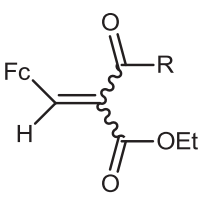
We found that the reaction of ethyl 2-acyl-3-ferrocenylacrylates **2a**–**d** with guanidinium carbonate (**3a**) and 1,1-dimethylguanidinium sulfate (**3b**) in aqueous ethanol in the presence of Na_2CO_3 at *ca.* 80–85 °C affords ethyl 2-amino-6-ferrocenyl-1,6-dihydropyrimidine-5-carboxylates (**4a**–**f**) (*ca.* 35–42%) along with some other condensation and decomposition products of the starting acrylates (Scheme 2). Studies of their compositions and structures are currently in progress.

Ferrocenyldihydropyrimidines were isolated by column chromatography on alumina as orange substances stable on storage in solid state. The structures of compounds **4a**–**f** were established based on the data from IR spectroscopy, ^{1}H and ^{13}C NMR spectroscopy, mass spectrometry, and elemental analysis.

Thus the ^{1}H NMR spectra of the dihydropyrimidines contain singlets for the methine protons of the FcCH-fragments and broadened singlets for the NH protons. The protons for the substituted cyclopentadienyl ring of ferrocene appear as four multiplets located in a higher field compared to the singlets of the unsubstituted cyclopentadienyl ring. The ^{1}H NMR spectra of compounds **4a**–**f** contain also the signals for the methyl, aryl, amino and ethoxycarbonyl substituents.

The ^{13}C NMR spectra of these compounds corroborate completely their 1,6-dihydropyrimidine structure. They contain signals for CipsoFc at δ *ca.* 90–94 ppm; four signals for the carbon atoms of the C_5H_4 ring appear upfield compared to the singlet of the carbon atoms of the C_5H_5 ring. The methylene carbon atoms of the $-CO_2Et$ substituents resonate in the region of δ *ca.* 59–62 ppm.

We found further that ferrocenyldihydropyrimidines **4a**–**f** underwent smooth oxidative dehydrogenation with diacetoxyiodobenzene in dichloromethane in the presence of K_2CO_3 (*ca.* 20–30 min (**4a**–**c**) or ~40 min (**4d**–**f**), room temperature) to afford the corresponding pyrimidines (**5a**–**f**) in *ca.* 65–75% yields (see Scheme 2).



2a-d

R= CH_3 (**2a**, **2b**); R= Ph (**2c**); R= 4-$C_6H_4NO_2$ (**2d**)

Fig. 1. Structures of the starting compounds.

Ethyl 2-amino-6-ferrocenylpyrimidine-5-carboxylates **5a**–**f** isolated by column chromatography on alumina represent red substances stable on storage in solid state and in solutions (*e.g.*, in dichloromethane, ethanol, and acetone).

The structures of these products were established based on the data from IR spectroscopy, ^{1}H and ^{13}C NMR spectroscopy, mass spectrometry, and elemental analysis. The principal NMR spectral features of pyrimidines **5a**–**f** are as follows. The signals for the four protons of the C_5H_4 ring of ferrocene appear as two multiplets located in a lower field compared to the singlets of the unsubstituted cyclopentadienyl fragments. The ^{13}C NMR spectra contain two singlets for four carbon atoms of the substituted cyclopentadienyl fragments of ferrocene, one at a lower field and the other at a higher field compared with the position of the singlet for the five carbon atoms of the unsubstituted cyclopentadienyl fragments of ferrocene. The signals for the CipsoFc, CH_3 groups, and ethoxycarbonyl substituents are upfield shifted compared to the corresponding signals of amino(ferrocenyl)dihydropyrimidines **4a**–**f**.

The systematic comparative analysis of the ^{1}H and ^{13}C NMR spectra of dihydropyrimidine and pyrimidine derivatives, **4a**–**f** and **5a**–**f**, reveals the effect of aromatization. The distinct differences in the NMR characteristics make it possible to use them for the identification of these types of compounds from their ^{1}H and ^{13}C NMR spectra (Fig. 2).

The spatial structures of compounds **4a**, **4e**, and **5e** were determined by X-ray diffraction of their single crystals. Crystals of **4a** were obtained by crystallization from dimethyl sulfoxide, crystals of **4e** and **5e** were obtained by crystallization from dichloromethane and chloroform, respectively.

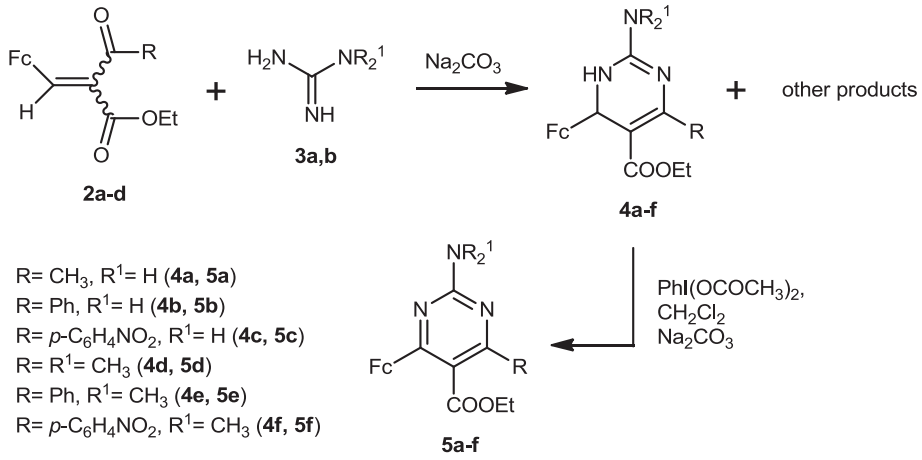
The general views of molecules **4a**, **4e**, and **5e** are shown in Figs. 3–5, respectively, the principal geometric parameters are listed in Table 1. The data from X-ray diffraction analysis confirmed the dihydropyrimidine structures for compounds **4a** and **4e** and an aromatic structure for compound **5e**.

The central fragment of the molecule **5e** is a flat six-membered ring with two nitrogen atoms. Data from the X-ray analysis show that the C(17)–C(18) bond in the pyrimidine ring is somewhat longer [d = 1.408(2) Å] than the standard value of 1.38 Å (Ref. [1]). The lengths of the C–Fe and C–C bonds in the ferrocenyl substituent as well as the geometric parameters of the ferrocene sandwich are close to the standard values [29] (Table 2).

The following putative reaction scheme seems to rationalize the formation of 1,6-dihydropyrimidines **4a**–**f** (Scheme 3). The two nucleophilic sites of guanidines **3a**, **b** attack sequentially or simultaneously the carbon atoms in positions 3 of ethyl 2-acyl-3-ferrocenylacrylates **2a**–**d** and the carbon atoms of the carbonyl groups of the acyl substituents followed by dehydration of intermediates (**6a**–**f**).

The mechanism for the oxidative dehydrogenation with a $PhI(OAc)_2/K_2CO_3$ system proposed by Richardson et al. [30] seems to be applicable for compounds **4a**–**f** (Scheme 4).

Initially, $PhI(OAc)_2$ reacts with the dihydropyrimidines to form *N*(1)-[acetoxy(phenyl)-λ^3-iodo]-derivatives (**7a**–**f**). Their transformations to the target pyrimidines **5a**–**f** occurs presumably *via* intermediates (**8a**–**f**) with elimination of PhI. The oxidative dehydrogenation of dihydropyrimidines **4a**–**f** proceeds fast and is not virtually accompanied by the formation of side products. However, the reduction of the yields of pyrimidines **5a**–**c** with the free amino group is observed even upon insignificant increase in the reaction time (the maximum duration is 30 min), presumably, due to the involvement of the free amino group in the oxidation (the formation of nitrenes and their subsequent transformations).



Scheme 2.

3. Pharmacology

In order to examine the applicability of four types of compounds (**4a**–**c**, **4d**–**f**, **5a**–**c** and **5d**–**f**) as antitumor agents, they were tested *in vitro* against six human tumor cell lines: U-251 (glioma), PC-3 (prostate), K-562 (leukemia), HCT-15 (colon), MCF-7 (breast) and SKLU-1 (lung). A primary screening at a fixed concentration of 50 μM showed cytotoxicity against the six human tumor cell lines

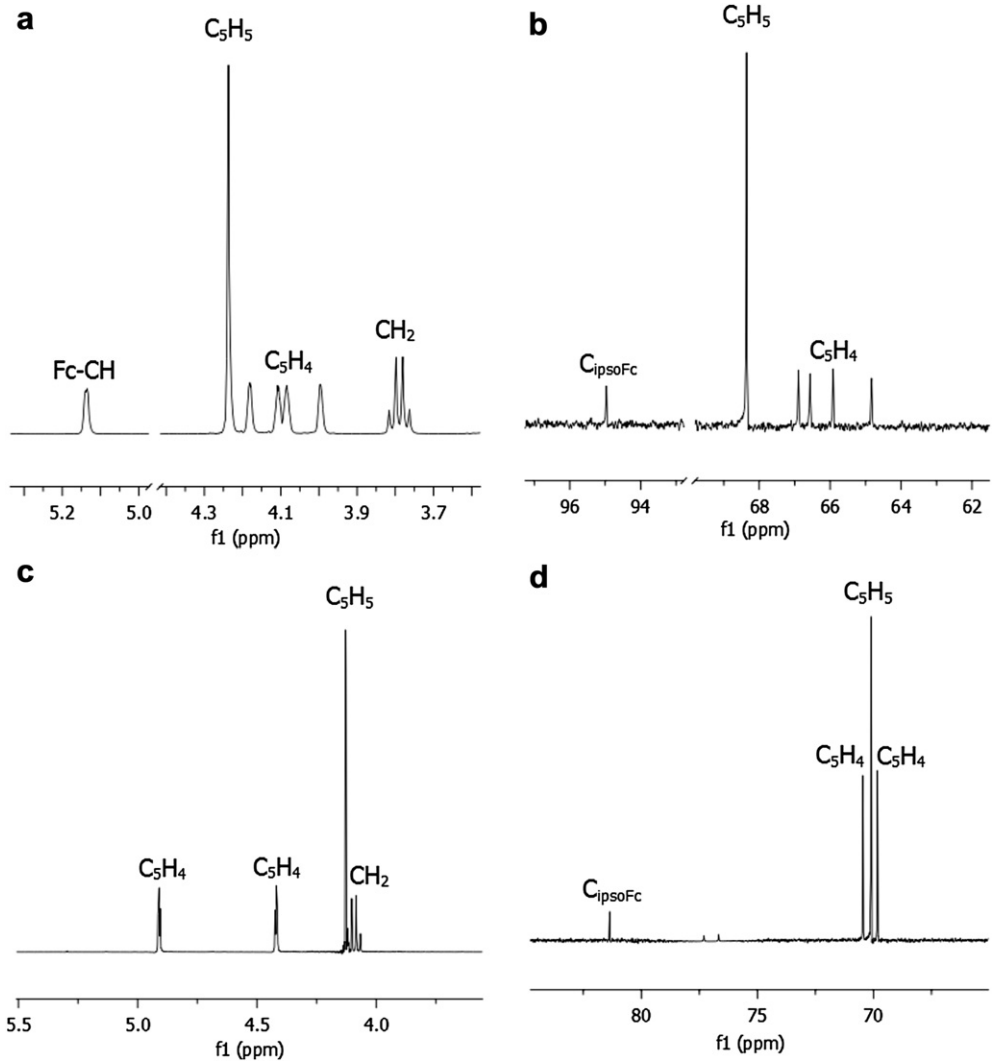


Fig. 2. Characteristic fragments of ^{1}H and ^{13}C NMR spectra: (a) and (b) of **4b**, respectively; (c) and (d) of **5e**.

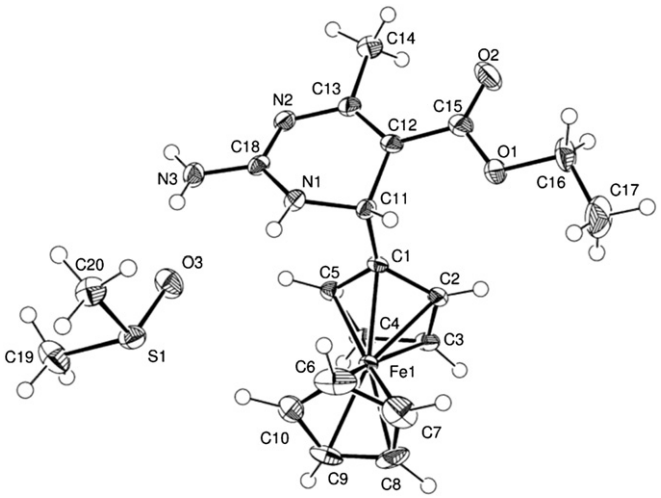


Fig. 3. X-ray crystal structure of **4a**.

tested. Cisplatin was used at the same concentration as a positive control. Peritoneal mouse macrophages (MØ) were also determined at 50 μM in DMSO (Table 3).

Compounds **4b** and **4c** showed a 100 percent inhibition of cellular growth at 50 μM for six human tumor cell lines, compounds **4a** and **4d** showed better activity than cisplatin for K-562. In addition, the IC_{50} values for these compounds were determined and the results are summarized in Table 4.

The cytotoxic *in vitro* activity of the ferrocene derivatives varied from $IC_{50} > 100$ μM (in the case of PC-3 and K-562 cancer cell lines) for **5e** and **5f** to $IC_{50} = 6.6$ μM (PC-3) and $IC_{50} = 8.5$ μM (K-562) for **4c** and $IC_{50} = 8.7$ μM (PC-3) and $IC_{50} = 7.4$ μM (K-562) for **4b**. These values, being lower than those for cisplatin ($IC_{50} = 15.9$ μM for PC-3 and $IC_{50} = 15.2$ μM for K-562), make compounds **4c** and **4b** as promising antiproliferative agents against two human tumor cell lines: PC-3 (prostate) and K-562 (leukemia).

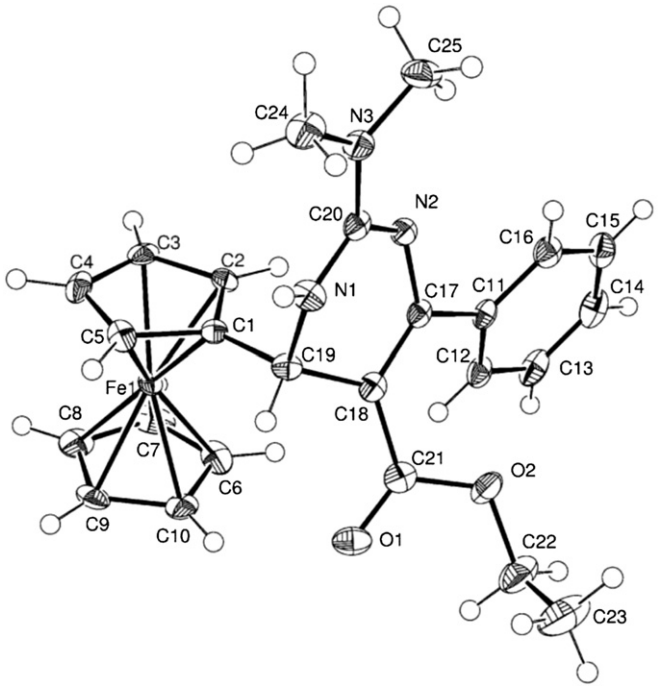


Fig. 4. . X-ray crystal structure of **4e**.

4. Conclusion

The six novel 2-amino-6-ferrocenyl-1,6-dihydropyrimidine-5-carboxylates **4a**–**f** and six novel 2-amino-6-ferrocenylpyrimidine-5-carboxylates **5a**–**f** were prepared and structurally characterized using spectroscopic techniques. Most of synthesized compounds displayed modest cytotoxic activity in the micromolar range. Compounds containing 1,6-dihydropyrimidyl moiety **4a**–**f** appeared to be the most active against six human tumor cell lines than the pyrimidine-5-carboxylates **5a**–**f**. Compounds **4b** and **4c** exhibited the highest activity against almost tumoral cell lines *y* cisplatin, which was used as reference. These results identified that ferrocenyl(dihydro)pyrimidines are new leads in antitumor chemotherapy. The study suggest the beneficial potential of these leads that need to be further explored in order to discover and develop better and yet safety therapeutic antitumor agents.

5. Experimental

All the solvents were dried according to standard procedures [31] and were freshly distilled before use. Column chromatography and TLC were carried out on alumina (Brockmann activity III). The ^1H and ^{13}C NMR spectra were recorded on a Unity Inova Varian spectrometer (300 and 75 MHz, compounds **4d**–**f**, **5b**,**c**) and Varian 400-MR spectrometer (400 and 100 MHz, compounds **4a**–**c**, **5a**, **5d**–**f**) for solutions in $CDCl_3$ and DMSO-d_6, with Me_4Si as the internal standard. The IR spectra were measured with an FTIR spectrophotometer (Spectrum RXI Perkin–Elmer instruments) using KBr pellets. UV spectra were recorded on a Specord UV–VIS spectrophotometer. 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.

The unit cell parameters and the X-ray diffraction intensities of **4a**, **4e** and **5e** were recorded on a Gemini (detector Atlas CCD, Cryojet N_2) diffractometer. The structure of compounds **4a**, **4e** and **5e** were solved by the direct method (SHELXS-97 [32]) and refined using full-matrix least-squares on F^2.

The following reagents were purchased from Aldrich: ferrocenecarbaldehyde, 99%; ethyl acetoacetate, 99+%; ethyl benzoylacetate, 90%; ethyl 4-nitrobenzoylacetate; guanidinium carbonate, 99%; 1,1-dimethylguanidinium sulfate, 97%; diacetoxyiodobenzene, 98%.

Ethyl 2-acyl-3-ferrocenylacrylates **2a**–**d** were prepared by condensation of ferrocenecarbaldehyde with ethyl acetoacetate, ethyl benzoylacetate and ethyl 4-nitrobenzoylacetate, respectively, in benzene in the presence of piperidinium acetate. The physical and ^1H NMR spectroscopic characteristics of compounds **2a**, **2b**, **2c** and **2d** were in accord with the literature data [27,33,34].

5.1. Synthesis

5.1.1. Reactions of ethyl 2-acyl-3-ferrocenylacrylates *2a–d* with guanidinium carbonate (*3a*) (general procedure)

A mixture of compound **2a** (**2b**–**d**) (10 mmol), guanidinium carbonate **3a** (1.35 g, 7.5 mmol), ethanol (60 ml), H_2O (10 ml) and 2.0 g Na_2CO_3 was stirred for 8 h at 80 °C. The solvents were removed *in vacuo* and the residue was dissolved in dichloromethane (50 ml). The solution was mixed with Al_2O_3 (activity III) (20 g) and the solvent was evaporated in air. This sorbent was applied onto a column with Al_2O_3 (the height of alumina is *ca.* 20 cm) and the reaction products were eluted from the column first with petroleum ether, then with a 1:2 dichloromethane – petroleum ether and 1:10 methanol – dichloromethane solvent system to give dihydropyrimidines **4a**–**c**.

Ethyl 2-amino-6-ferrocenyl-4-methyl-1,6-dihydropyrimidine-5-carboxylate (**4a**), orange crystals, yield 0.74 g (40%, from **2a**),

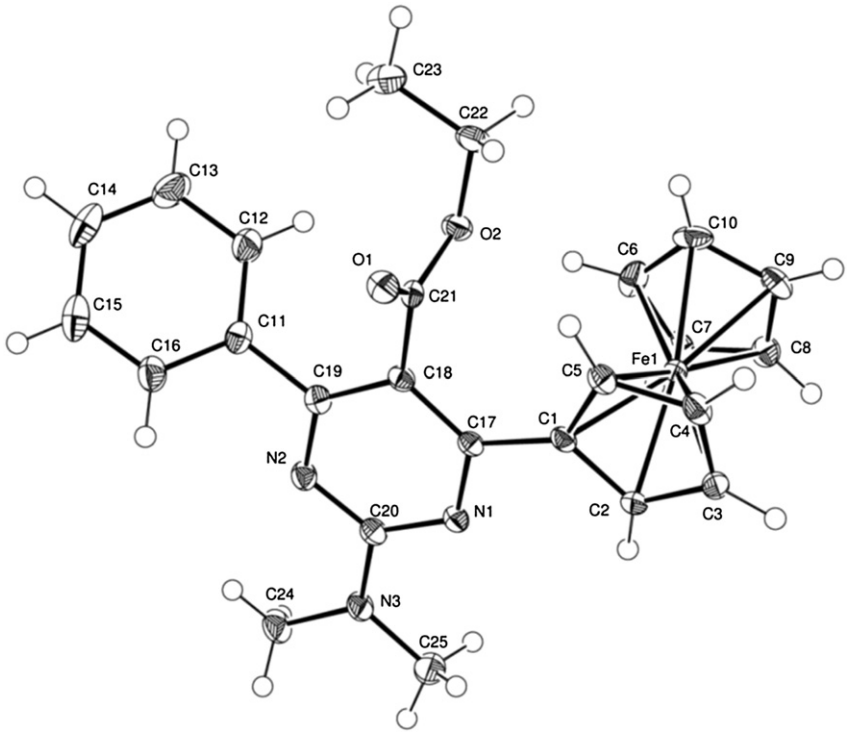


Fig. 5. X-ray crystal structure of **5e**.

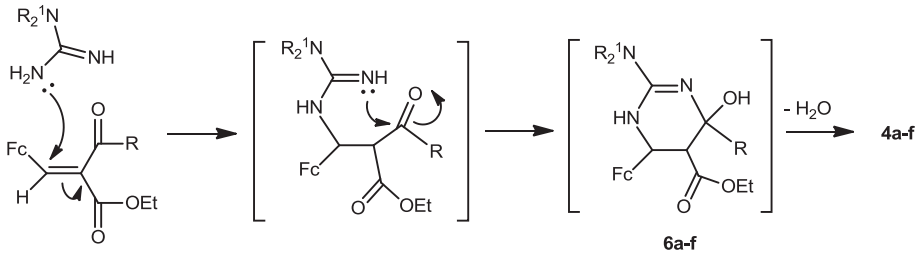
0.77 g (42%, from **2b**), m.p. 126–128 °C. IR (KBr): ν 486, 502, 607, 771, 803, 821, 953, 1004, 1020, 1052, 1077, 1104, 1152, 1198, 1219, 1306, 1338, 1371, 1480, 1571, 1667, 1721, 2252, 2767, 2900, 2980, 3324 cm^{-1} ^1H NMR (400 MHz, DMSO-d_6): δ 1.32 (3H, t, CH_3, $J = 7.2$ Hz), 2.32 (3H, s, CH_3), 4.20 (2H, q, CH_2, $J = 7.2$ Hz), 4.28 (5H, s, C_5H_5), 4.01 (1H, m, C_5H_4), 4.11 (1H, m, C_5H_4), 4.13 (1H, m, C_5H_4), 4.22 (1H, m, C_5H_4), 5.20 (1H, s, CH), 5.33 (1H, bs, NH), 7.16 (2H, bs, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.32, 21.79 ($2CH_3$), 47.57 (CH), 59.49 (CH_2), 68.62 (C_5H_5), 64.66, 66.09, 67.26, 67.51 (C_5H_4), 92.21 (C_{ipso}Fc), 102.60, 153.14, 162.12, 165.17 (4C). Anal. Calcd. for $C_{18}H_{21}FeN_3O_2$: C, 58.87; H, 5.76; Fe, 15.21; N, 11.44. Found: C, 58.72; H, 5.83; Fe, 15.34; N, 11.29%. MS: *m*/*z* 367 $[M]^+$.

Table 1
Selected bond lengths and angles for compounds **4a**, **4e**, and **5e**.

Bond lengths [Å]		Bond angles [°]	
4a			
N(1)–H(1N)	0.82(3)	N(1)–C(11)–H(11)	108.9
C(11)–H(11)	1.0000	C(18)–N(1)–H(1N)	115.0 (19)
N(1)–C(11)	1.463(2)	N(1)–C(18)–N(2)	123.04(17)
N(1)–C(18)	1.348(2)	C(18)–N(2)–C(13)	117.07 (16)
N(2)–C(18)	1.332(3)	N(2)–C(13)–C(12)	122.92(18)
C(12)–C(13)	1.370(3)	C(13)–C(12)–C(11)	117.89(17)
C(12)–C(11)	1.525(3)	C(12)–C(11)–N(1)	107.27(15)
N(2)–C(13)	1.376(3)	C(11)–N(1)–C(18)	119.45(16)
N(3)–C(18)	1.336(3)	N(1)–C(18)–N(3)	117.98(18)
4e			
N(1)–H(1N)	0.85(3)	N(1)–C(19)–H(19)	109.3
H(19)–C(19)	1.0000	C(20)–N(1)–H(1N)	123 (2)
N(1)–C(19)	1.476(3)	C(18)–C(17)–N(2)	122.4(2)
N(1)–C(20)	1.342(3)	C(19)–C(18)–C(17)	114.3(2)
N(2)–C(20)	1.342(3)	N(1)–C(19)–C(18)	105.49(18)
N(2)–C(17)	1.360(3)	C(17)–N(2)–C(20)	116.5(2)
N(3)–C(20)	1.341(3)	N(2)–C(20)–N(3)	118.4(2)
C(17)–C(18)	1.388(3)	N(3)–C(20)–N(1)	119.4(2)
C(18)–C(19)	1.511(3)	N(2)–C(20)–N(1)	122.2(2)
5e			
N(1)–C(20)	1.346(2)	C(18)–C(17)–N(1)	121.29(15)
N(1)–C(17)	1.343(2)	C(17)–C(18)–C(19)	116.59(15)
N(2)–C(20)	1.347(2)	N(1)–C(20)–N(2)	125.84(15)
N(2)–C(19)	1.336(2)	C(19)–N(2)–C(20)	116.46(14)
N(3)–C(20)	1.354(2)	N(2)–C(19)–C(18)	122.56(15)
C(17)–C(18)	1.408(2)	N(3)–C(20)–N(1)	116.66(15)
C(18)–C(19)	1.399(2)	N(2)–C(20)–N(3)	117.50(15)

Table 2
Crystal data and structure refinement parameters for compounds **4a**, **4e** and **5e**.

Data	**4a**	**4e**	**5e**
Molecular formula	$C_{18}H_{21}FeN_3O_2 \cdot (CH_3)_2SO$	$C_{25}H_{27}FeN_3O_2$	$C_{25}H_{25}FeN_3O_2$
Formula weight (g mol^{-1})	445.36	457.35	455.33
Temperature (K)	130(2)	130(2)	130(2)
Crystal system	Monoclinic	Triclinic	Monoclinic
Space group	P21/c	P-1	P21/c
a(Å)	10.0339(5)	10.3102(7)	7.3017(2)
b(Å)	19.6886(7)	10.7919(7)	26.4303(6)
c(Å)	10.8598(5)	11.6861(8)	10.8992(2)
α(°)	90	115.227	90
β(°)	103.751(4)	111.697(6)	97.251(2)
γ(°)	90	92.325(5)	90
V($Å^3$)	2083.90(16)	1062.13(15)	2086.57(8)
Z	4	2	4
D calc. (mg/m^3)	1.420	1.430	1.449
Wavelength (Å)	1.54180	1.54184	0.71073
Absorption coefficient (mm^{-1})	6.952	5.911	0.751
F(000)	936	480	952
θ range (°)	4.49–68.08	4.63–67.82	3.54–26.06
Reflections collected	13,559	6432	14,970
Reflections independent	3799	3833	4110
R_{int}	0.0271	0.0328	0.0244
Final *R* indices [$I > 2\sigma(I)$]	$R_1 = 0.0306$ $wR_2 = 0.0825$	$R_1 = 0.0400$ $wR_2 = 0.1106$	$R_1 = 0.0286$ $wR_2 = 0.0691$
R indices (all data)	$R_1 = 0.0338$ $wR_2 = 0.0834$	$R_1 = 0.0440$ $wR_2 = 0.1127$	$R_1 = 0.0345$ $wR_2 = 0.0708$
Refinable parameters	266	288	283
Goodness-of-fit on F^2	1.065	1.076	1.027
Maximum/minimum residual electron density (e$Å^{-3}$)	0.326/−0.324	0.788/−0.347	0.353/−0.311



Scheme 3.

Ethyl 2-amino-6-ferrocenyl-4-phenyl-1,6-dihydropyrimidine-5-carboxylate (**4b**), orange crystals, yield 0.95 g (40%), m.p. 198–199 °C. IR (KBr): *ν* 483, 511, 621, 780, 812, 821, 973, 1001, 1019, 1042, 1083, 1101, 1161, 1200, 1223, 1314, 1337, 1392, 1475, 1548, 1573, 1682, 1716, 2259, 2781, 2910, 3004, 3341 cm^{-1} ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.83 (3H, t, CH_3, $J = 6.9$ Hz), 3.76 (2H, q, CH_2, $J = 6.9$ Hz), 4.23 (5H, s, C_5H_5), 3.99 (1H, m, C_5H_4), 4.06 (1H, m, C_5H_4), 4.09 (1H, m, C_5H_4), 4.18 (1H, m, C_5H_4), 5.12 (1H, s, CH), 6.42 (1H, bs, NH), 7.10 (2H, bs, NH_2), 7.16 (2H, m, C_6H_5), 7.22 (3H, m, C_6H_5). ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.89 (CH_3), 47.82 (CH), 56.46 (CH_2), 69.04 (C_5H_5), 66.01, 66.66, 67.93, 68.29 (C_5H_4), 89.18 (C_{ipso}Fc), 121.05, 126.99, 128.72 (C_6H_5), 99.13, 134.87, 147.42, 157.84, 160.11 (5C). Anal. Calcd. for $C_{23}H_{23}FeN_3O_2$: C, 64.35; H, 5.40; Fe, 13.01; N, 9.79%. Found: C, 64.48; H, 5.29; Fe, 13.12; N, 9.82%. MS: *m*/*z* 429 $[M]^+$.

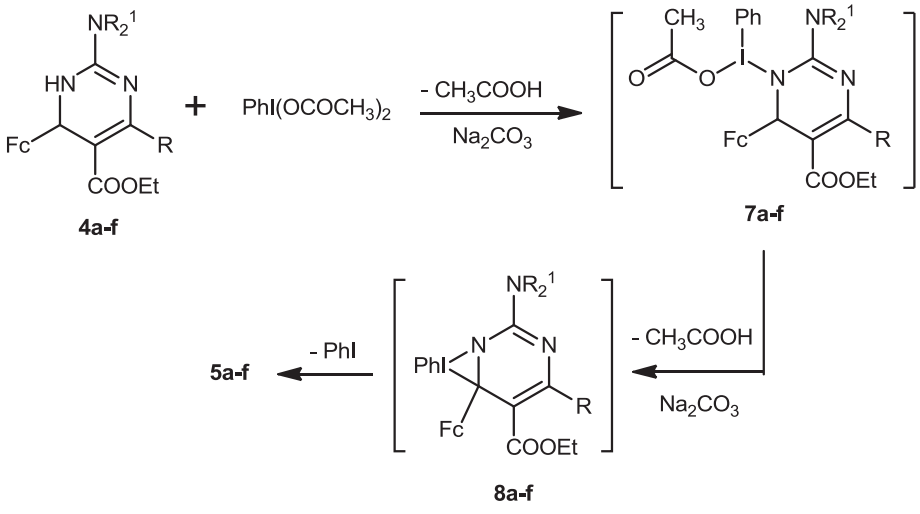
Ethyl 2-amino-6-ferrocenyl-4-(4-nitrophenyl)-1,6-dihydropyrimidine-5-carboxylate (**4c**), orange crystals, yield 0.95 g (40%), m.p. 195–196 °C. IR (KBr): *ν* 486, 503, 609, 784, 806, 821, 969, 1002, 1021, 1039, 1080, 1105, 1152, 1202, 1217, 1315, 1337, 1389, 1479, 1547, 1570, 1678, 1712, 2264, 2779, 2908, 3006, 3325 cm^{-1} ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.85 (3H, t, CH_3, $J = 7.2$ Hz), 3.80 (2H, q, CH_2, $J = 7.2$ Hz), 4.23 (5H, s, C_5H_5), 3.99 (1H, m, C_5H_4), 4.07 (1H, m, C_5H_4), 4.09 (1H, m, C_5H_4), 4.19 (1H, m, C_5H_4), 5.14 (1H, s, CH), 6.56 (1H, bs, NH), 7.20 (2H, bs, NH_2), 7.38 (2H, d, C_6H_4, $J = 8.7$ Hz), 8.13 (2H, d, C_6H_4, $J = 8.7$ Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.83 (CH_3), 48.01 (CH), 58.54 (CH_2), 68.45 (C_5H_5), 65.02, 65.83, 67.45, 67.57 (C_5H_4), 94.28 (C_{ipso}Fc), 122.65, 129.75 (C_6H_4), 99.46, 146.29, 149.75, 156.03, 158.04, 165.68 (6C). Anal. Calcd. for $C_{23}H_{22}FeN_4O_4$: C, 58.25; H, 4.68; Fe, 11.77; N, 11.80%. Found: C, 58.34; H, 4.57; Fe, 11.87; N, 11.69%. MS: *m*/*z* 474 $[M]^+$.

*5.1.2. Reactions of ethyl 2-acyl-3-ferrocenylacrylates **2a–d** with 1,1-dimethylguanidine **3b***

Following the general procedure, the reactions of **2a–d** (5 mmol) with 1,1-dimethylguanidinium sulfate **3b** (2.04 g, 7.5 mmol) in a mixture of ethanol (70 ml) and water (10 ml) in the presence of Na_2CO_3 (2.5 g) (6 h at 80 °C) afforded compounds **4d**, **4e**, and **4f**.

Ethyl 2-dimethylamino-6-ferrocenyl-4-methyl-1,6-dihydropyrimidine-5-carboxylate (**4d**), orange crystals, yield 0.87 g (45%, **2a**), 0.81 g (41%, from **2b**), m.p. 145–146 °C. IR (KBr): *ν* 495, 796, 826, 947, 1000, 1068, 1098, 1120, 1183, 1208, 1231, 1245, 1337, 1373, 1477, 1563, 1640, 1724, 2903, 2938, 2976, 3077, 3091, 3333 cm^{-1} ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.30 (3H, t, CH_3, $J = 7.2$ Hz), 2.28 (3H, s, CH_3), 3.15 (6H, s, $2CH_3$), 4.17 (2H, q, CH_2, $J = 7.2$ Hz), 4.13 (5H, s, C_5H_5), 3.94 (1H, m, C_5H_4), 4.01 (1H, m, C_5H_4), 4.08 (1H,m, C_5H_4), 4.26 (1H, m, C_5H_4), 5,21 (1H, s, CH), 5.38 (1H, bs, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.66, 24.25, 36.96 ($4CH_3$), 49.32 (CH), 59.12 (CH_2), 68.26 (C_5H_5), 64.95, 67.26, 67.34, 67.67 (C_5H_4), 94.41 (C_{ipso}Fc), 99.92, 154.32, 158.43, 167.46 (4C).Anal. Calcd. for $C_{20}H_{25}FeN_3O_2$: C, 60.77; H, 6.38; Fe, 14.13; N, 10.63. Found: C, 60.61; H, 6.49; Fe, 14.25; N, 10.58%. MS: *m*/*z* 395 $[M]^+$.

Ethyl 2-dimethylamino-6-ferrocenyl-4-phenyl-1,6-dihydropyrimidine-5-carboxylate (**4e**), orange crystals, yield 0.92 g (40%), m.p. 159–160 °C. IR (KBr): *ν* 491, 699, 778, 808, 816, 999, 1001, 1021, 1037, 1055, 1089, 1152, 1217, 1282, 1325, 1344, 1522, 1571, 1620, 1687,1737, 2933, 2975, 3074, 3303 cm^{-1} ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.9 (3H, t, CH_3, $J = 6.9$ Hz), 3.11 (6H, s, $2CH_3$), 3.91 (2 H, q, CH_2, $J = 6.9$ Hz), 4.19 (5H, s, C_5H_5), 4.06 (2H, m, C_5H_4), 4.14 (1H, m, C_5H_4), 4.38 (1H, m, C_5H_4), 5.32 (1H, s, CH), 5.54 (1H, s, NH), 7.30 (3H, m,



Scheme 4.

Table 3
Inhibition of human tumor cells lines by compounds (**4a**–**f**, **5a**–**f**) and peritoneal mouse macrophages (MØ) at 50 μM in DMSO.[a]

Compounds	% of growth inhibition						
	U-251	PC-3	K-562	HCT-15	MCF-7	SKLU-1	MØ
4a	65.4 ± 4.6	75.3 ± 1.1	94.6 ± 5.4	68.9 ± 15.0	77.7 ± 6.7	59.6 ± 4.4	35.6 ± 4.7
4b	100	100	100	100	100	100	34.0 ± 2.8
4c	100	100	100	100	100	100	72.5 ± 3.1
4d	51.8 ± 5.7	89.7 ± 10.2	91.3 ± 8.7	30.2 ± 4.4	78.9 ± 7.6	40.5 ± 3.6	71.5 ± 2.9
4e	42.1 ± 6.5	71.9 ± 10.3	53.85 ± 2.1	35.4 ± 11.8	42.9 ± 12.0	44.8 ± 4.1	69.5 ± 4.2
4f	57.8 ± 6.6	71.8 ± 28.2	69.3 ± 6.1	62.0 ± 10.1	63.4 ± 6.6	36.6 ± 15.4	31.5 ± 0.3
5a	31.6 ± 3.5	81.1 ± 18.9	64.3 ± 9.3	77.9 ± 17.9	74.3 ± 9.2	38.9 ± 3.6	24.5 ± 4.5
5b	37.7 ± 5.0	63.9 ± 13.0	64.1 ± 10.5	54.98 ± 9.7	40.98 ± 4.7	46.74 ± 4.3	36.6 ± 3.4
5c	65.3 ± 1.7	65.0 ± 12.2	56.6 ± 2.1	67.3 ± 0.2	39.8 ± 0.005	50.5 ± 1.3	42.9 ± 1.6
5d	43.8 ± 8.0	52.3 ± 6.9	72.4 ± 4.6	44.6 ± 4.3	56.1 ± 12.5	43.6 ± 4.2	34.1 ± 5.3
5e	15.5 ± 0.5	37.4 ± 2.3	15.65 ± 0.8	29.5 ± 1.2	42.2 ± 4.0	38.4 ± 7.6	31.3 ± 4.3
5f	11.0 ± 1.9	32.4 ± 0.1	12.1 ± 4.7	14.8 ± 2.7	34.0 ± 2.9	25.3 ± 3.2	26.2 ± 1.3
Cisplatin	89.9 ± 8.1	86.7 ± 4.1	74.4 ± 2.1	81.8 ± 7.1	77.9 ± 2.5	95.8 ± 2.1	22.9 ± 5.9

[a] Results express mean ± standard error (SEM) obtained from **2** independent experiments performed at 48 h.

C_6H_5), 7.39(2H, m, C_6H_5). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.98, 37.30 ($3CH_3$), 50.17 (CH), 59.28 (CH_2), 68.49 (C_5H_5), 65.32, 67.50, 67.66, 68.04 (C_5H_4), 93.84 (C_{ipso}Fc), 127.24, 127.96, 128.92 (C_6H_5), 100.03, 129.06, 142.13, 154.42, 167.64 (5C). Anal. Calcd. for $C_{25}H_{27}FeN_3O_2$: C, 65.66; H, 5.95; Fe, 12.21; N, 9.18. Found: C, 65.53; H, 6.07; Fe, 12.30; N, 9.06%. MS: *m*/*z* 457 $[M]^+$.

Ethyl 2-dimethylamino-6-ferrocenyl-4-(4-nitrophenyl)-1,6-dihydropyrimidine-5-carboxylate (**4f**), orange crystals, yield 0.88 g (35%), m.p. 158–159 °C. IR (KBr): ν 486, 530, 708, 757, 812, 861, 965, 1002, 1021, 1033, 1083, 1105, 1152, 1226, 1287, 1335, 1363, 1387, 1470, 1515, 1570, 1658, 1718, 2940, 3096, 3225 cm^{-1} ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.93 (3H, t, CH_3, $J = 6.9$ Hz), 3.16 (6 H, s, $2CH_3$), 3.92 (2H, q, CH_2, $J = 6.9$ Hz), 4.19 (5H, s, C_5H_5), 4.05 (1H, m, C_5H_4), 4.10 (1H, m, C_5H_4), 4.17 (1H, m, C_5H_4), 4.37 (1H, m, C_5H_4), 5.33 (1H, s, CH), 5.48 (1H, bs, NH), 7.49 (2H, d,C_6H_4, $J = 8.7$ Hz), 8.15 (2H, d, C_6H_4, $J = 8.7$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.09, 37.26 (3 CH_3), 50.03 (CH), 59.56 (CH_2), 68.51 (C_5H_5), 67.51, 67.84, 68.23, 68.92 (C_5H_4), 93.58 (C_{ipso}Fc), 122.65, 129.75 (C_6H_4), 100.57, 127.09, 147.29, 149.45, 154.51, 166.68 (6C). Anal. Calcd. for $C_{25}H_{26}FeN_4O_4$: C, 59.77; H, 5.22; Fe, 11.12; N, 11.15. Found: C, 59.65; H, 5.27; Fe, 11.08; N, 11.20%. MS: *m*/*z* 502 $[M]^+$.

5.1.3. Reactions of ethyl 2-dimethylamino-6-ferrocenyl-4-methyl(or -aryl)-1,6-dihydropyrimidine-5-carboxylates ***4a–f*** with diacetoxyiodobenzene

A mixture of compounds **4a**–**f** (3 mmol), diacetoxyiodobenzene (1.3 g, 4 mmol), dichloromethane (60 ml), and 1.5 g Na_2CO_3 was stirred for 1 h at 20 °C. The mixture was mixed with Al_2O_3 (activity III) (10 g) and the solvent was evaporated in air. This sorbent was applied onto a column with Al_2O_3 (the height of alumina is *ca.* 20 cm) and the reaction products were eluted from the column with a 1:2 ether – petroleum ether solvent system to give compounds **5a**–**f**, respectively.

Table 4
Inhibitory concentration[a] (IC_{50} μM) values obtained in PC-3 and K-562 cell lines for compounds **4a**–**f** and **5a**–**f** in DMSO.

Compound	Cell line IC_{50} (μM)[a]	
	PC-3	K-562
4a	12.7 ± 1.1	38.3 ± 1.6
4b	8.7 ± 0.7	7.4 ± 0.1
4c	6.6 ± 0.5	8.5 ± 0.2
4d	27.3 ± 2.4	21.0 ± 1.2
4e	48.2 ± 3.7	55.5 ± 5.3
4f	43.8 ± 4.0	21.6 ± 2.0
5a	64.9 ± 5.9	34.0 ± 0.5
5b	49.4 ± 1.4	24.5 ± 2.3
5c	17.1 ± 1.5	18.0 ± 0.9
5d	31.2 ± 2.1	31.0 ± 1.5
5e	>100	>100
5f	>100	>100
Cisplatin	15.9 ± 1.2	15.2 ± 1.4

[a] Results express the mean IC_{50} ± standard error (SEM) obtained from three independent experiments performed at 48 h.

Ethyl 2-amino-6-ferrocenyl-4-methylpyrimidine-5-carboxylate (**5a**), red crystals, yield 1.4 g (70%), m.p. 120–121 °C. IR (KBr): ν 491, 793, 820, 923, 1002, 1061, 1086, 1120, 1181, 1200, 1233, 1232, 1331, 1368, 1472, 1565, 1642, 1719, 2931, 2934, 2971, 3065, 3018, 3321 cm^{-1} ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.34 (3H, t, CH_3, $J = 7.2$ Hz), 2.34 (3H, s, CH_3), 4.36 (2H, q, CH_2, $J = 7.2$ Hz), 4.11 (5H, s, C_5H_5), 4.40 (2H, m, C_5H_4), 4.80 (2H, m, C_5H_4), 5.32 (2H,bs, NH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.99, 22.18 ($2CH_3$), 61.47 (CH_2), 70.11 (C_5H_5), 69.29, 70.55 (C_5H_4), 80.38 (C_{ipso}Fc), 115.94, 161.52, 164.95, 165.28, 169.62 (5C). Anal. Calcd. for $C_{18}H_{19}FeN_3O_2$: C, 59.20; H, 5.24; Fe, 15.30; N, 11.50. Found: C, 59.27; H, 5.16; Fe, 15.43; N, 11.39%. MS: *m*/*z* 365 $[M]^+$.

Ethyl 2-amino-6-ferrocenyl-4-phenylpyrimidine-5-carboxylate (**5b**), red crystals, yield 1.8 g (72%), m.p. 149–150 °C. IR (KBr): ν 482, 531, 706, 760, 811, 858, 962, 1001, 1018, 1032, 1080, 1105, 1147, 1223, 1281, 1335, 1362, 1374, 1449, 1499, 1664, 1715, 2924, 3066, 3231 cm^{-1} ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.99 (3H, t, CH_3, $J = 7.2$ Hz), 4.10 (2H, q, CH_2, $J = 7.2$ Hz), 4.14 (5H, s, C_5H_5), 4.44 (2H, m, C_5H_4), 4.91 (2H, m, C_5H_4), 5. 23 (2H, bs, NH_2), 7.43 (3H, m, C_6H_5), 7.58 (2H, m, C_6H_5). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.79 (CH_3), 61.23 (CH_2), 69.67 (C_5H_5), 69.34, 70.35 (C_5H_4), 81.14 (C_{ipso}Fc), 128.17, 128.37, 129.15 (C_6H_5), 104.91, 139.64, 161.73, 165.43, 166.29, 169.81 (6C). Anal. Calcd. for $C_{23}H_{21}FeN_3O_2$: C, 64.65; H, 4.56; Fe, 13.07; N, 9.83. Found: C, 64.73; H, 4.47; Fe, 13.12; N, 9.71%. MS: *m*/*z* 427 $[M]^+$.

Ethyl 2-amino-6-ferrocenyl-4-(4-nitrophenyl)pyrimidine-5-carboxylate (**5c**), red crystals, yield 1.8 g (72%), m.p. 163–164 °C. IR (KBr): ν 477, 534, 711, 762, 812, 852, 960, 1002, 1021, 1038, 1079, 1100, 1148, 1229, 1291, 1339, 1363, 1395, 1472, 1523, 1568, 1661, 1718, 2942, 3096, 3229 cm^{-1} ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.06 (3H, t, CH_3, $J = 7.2$ Hz), 4.13 (2H, q, CH_2, $J = 7.2$ Hz), 4.15 (5H, s, C_5H_5), 4.48 (2H, m, C_5H_4), 4.91 (2H, m, C_5H_4), 5.18 (2H, bs, NH_2), 7.74 (2H, d, C_6H_4, $J = 8.4$ Hz), 8.30 (2H, d, C_6H_4, $J = 8.4$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.82 (CH_3), 61.92 (CH_2), 70.40 (C_5H_5), 69.88, 71.26 (C_5H_4), 79.96 (C_{ipso}Fc), 123.64, 129.35 (C_6H_4), 115.65, 137.60, 147.23, 154.87, 161.75, 163.15, 168.97 (7C). Anal. Calcd. for $C_{23}H_{20}FeN_4O_4$: C, 58.49; H, 4.27; Fe, 11.82; N, 11.86. Found: C, 58.57; H, 4.19; Fe, 11.74; N, 11.75%. MS: *m*/*z* 472 $[M]^+$.

Ethyl 2-dimethylamino-6-ferrocenyl-4-methylpyrimidine-5-carboxylate (**5d**), red crystals, yield 1.4 g (70%), m.p. 55–56 °C. IR (KBr): ν 482, 503, 596, 647, 730, 797, 812, 893, 1021, 1044, 1085, 1104, 1167, 1200, 1222, 1286, 1329, 1368, 1413, 1439, 1525, 1571, 1704, 2897, 2928, 2979, 3085 cm^{-1} ^{1}H NMR (400 MHz, $CDCl_3$):

δ 1.29 (3H, t, CH_3, $J = 7.2$ Hz), 2.36 (3H, s, CH_3), 3.24 (6H, s, $2CH_3$), 4.30 (2H, q, CH_2, $J = 7.2$ Hz), 4.08 (5H, s, C_5H_5), 4.35 (2H, m, C_5H_4), 4.80 (2H, m, C_5H_4). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.01, 22.67, 36.76 ($4CH_3$), 61.13 (CH_2), 70.01 (C_5H_5), 69.41, 69.93 (C_5H_4), 82.09 (C_{ipso}Fc), 113.16, 160.85, 164.11, 164.46, 170.26 (5C). Anal. Calcd. for $C_{20}H_{23}FeN_3O_2$: C, 61.10; H, 5.89; Fe, 14.20; N, 10.68. Found: C, 60.97; H, 5.94; Fe, 14.14; N, 10.73%. MS: m/z 393 $[M]^+$. Ethyl 2-dimethylamino-6-ferrocenyl-4-phenylpyrimidine-5-carboxylate (**5e**), red crystals, yield 1.55 g (68%), m.p. 114–116 °C. IR (KBr): ν 476, 497, 529, 641, 699, 761, 810, 821, 999, 1024, 1037, 1041, 1070, 1127, 1168, 1199, 1217, 1293, 1354, 1383, 1404, 1441, 1492, 1518, 1549, 1582, 1710, 2864, 2927, 2958, 3074, 3002 cm^{-1} ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.99 (3H, t, CH_3, $J = 7.2$ Hz), 3.29 (6H, s, 2 CH_3), 4.08 (2H, q, CH_2, $J = 7.2$ Hz), 4.13 (5H, s, C_5H_5), 4.38 (2H, m, C_5H_4), 4.93 (2H, m, C_5H_4), 7.41 (3H, m, C_6H_5), 7.63(2H, m, C_6H_5). ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.60, 36.86 ($3CH_3$), 61.20 (CH_2), 70.402 (C_5H_5), 69.85, 70.16 (C_5H_4), 81.92 (C_{ipso}Fc), 128.11, 128.17, 129.13 (C_6H_5), 112.96, 139.48, 160.89, 164.74, 164.85, 170.15 (6C). Anal. Calcd. for $C_{25}H_{25}FeN_3O_2$: C, 65.95; H, 5.53; Fe, 12.27; N, 9.22. Found: C, 70.06; H, 5.61; Fe, 12.34; N, 9.16%. MS: m/z 455 $[M]^+$.

Ethyl 2-dimethylamino-6-ferrocenyl-4-(4-nitrophenyl)pyrimidine-5-carboxylate (**5f**), red crystals, yield 1.8 g (72%), m.p. 156–157 °C. IR (KBr): ν 482, 503, 596, 730, 797, 812, 893, 1021, 1044, 1085, 1103, 1167, 1200, 1222, 1286, 1368, 1410, 1439, 1525, 1570, 1704, 2897, 2927, 2979, 3085 cm^{-1} ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.04 (3H, t, CH_3, $J = 7.2$ Hz), 3.29 (6H, s, $2CH_3$), 4.10 (2H, q, CH_2, $J = 7.2$ Hz), 4.13 (5H, s, C_5H_5), 4.42 (2H, m, C_5H_4), 4.91 (2H, m, C_5H_4), 7.79 (2H, d, C_6H_4, $J = 8.4$ Hz), 8.27 (2H, d, C_6H_4, $J = 8.4$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.68, 36.86 ($3CH_3$), 61.48 (CH_2), 70.10 (C_5H_5), 69.83, 70.45 (C_5H_4), 81.37 (C_{ipso}Fc), 123.31, 129.30 (C_6H_4), 112.75, 145.67, 148.14, 160.72, 162.41, 165.82, 169.54 (7C). Anal. Calcd. for $C_{25}H_{24}FeN_4O_4$: C, 60.02; H, 4.83; Fe, 11.16; N, 11.20. Found: C, 59.96; H, 4.76; Fe, 11.24; N, 11.11%. MS: m/z 500 $[M]^+$.

5.2. Biology

5.2.1. Cytotoxicity assay

The compound were screened *in vitro* against human cancer cell lines: HCT-15 (human colorectal adenocarcinoma), MCF-7 (human mammary adenocarcinoma), K-562 (human chronic myelogenous leukemia), U-251 (human glioblastoma), PC-3 (human prostatic adenocarcinoma), SKLU-1 (human lung adenocarcinoma), cell lines were supplied by National Cancer Institute (USA). The human tumor cytotoxicity was determined using the protein-binding dye sulforhodamine B (SRB) in microculture assay to measure cell growth, as described in the protocols established by the NCI (Monks et al. [35],) The cell lines were cultured in 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 and 25 μg/ml amphotericin B (Gibco) and 1% non-essential amino acids (Gibco). The cultures were maintained at 37 °C in a 5% CO_2 humidified atmosphere. As determined with trypan blue, the viability of the cell used en the experiments exceeded 95%.

The cells were removed from the tissue culture flasks by treatment with trypsin, and diluted with fresh media. One-hundred-microliters cell suspension aliquots, containing 5000–10,000 cell per well, were transferred into 96 well microtiter plates (Costar) and incubated at 37 °C for 24 h in a 5% CO_2 atmosphere.

Stock solutions of test compounds initially dissolved in DMSO (20 mM) were prepared and further diluted in medium to produce the desired concentrations. One-hundred-microliters aliquots of diluted solutions of test compounds were added to each well. The cultures were exposed for 48 h to the compound at concentrations 50 μM. After the incubation period, cells were fixed to the 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 trichloroacetic-acid-fixed cells were stained by the addition of 0.4% SRB. Free SRB solution was the removed by washing with 1% aqueous acetic-acid. The plates were the air-dried, and the bound dye was solubilized by the addition of 100 μl 10 mM-unbuffered Tris base. The plates were placed on a shaked for 5 min, prior analysis. Optical densities were determined in an Ultra Microplated Reader (El_x 808: Bio-Tek Instruments, Inc., Winooski, VT, USA) using test wavelength of 515 nm.

5.2.2. Isolation and culture of primary peritoneal macrophages

Isolation and culture of primary peritoneal macrophages were conducted as described elsewhere [36]. Swiss female mice, 25–30 g, were treated in accord with the Animal Care and Use Committee (NOM-062-Z00-1999). Mice were injected intraperitoneally with 1 ml of 3% (wt vol^{-1}) thioglycollate 3 days before harvesting. Peritoneal exudate cells were harvested, washed and suspended in DMEM. Peritoneal exudate cells were seeded into 48 well plates (Becton Dickinson, Oxnard, CA, USA) at a density of 1×10^6 cells ml^{-1}, and then incubated for 2 h at 37 °C in a 5% CO_2 incubator. Non-adherent cells were washed off and cultured in DMEM supplemented with 10% FCS.

Cell viability was determined by the MTT colorimetric assay. Briefly, 10 μl MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,3-diphenyltetrazolium bromide) was added to the medium after 48 h incubation with the test samples. After 4 h culture, the medium was removed and DMSO were added to dissolved the formazan solution produced in the cells. The optical density of the formazan solution was measured with a microplated reader at 414 nm [37].

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Appendix A. Supplementary material

CCDC 851259, 851260 and 851261 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.

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