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Original article

Synthesis, characterization and in vitro antiproliferative activities of new 13-*cis*-retinoyl ferrocene derivatives

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ABSTRACT

In order to improve biological behavior of the retinoyl derivatives, monoarylferrocenyl alcohols **9a** and **9b** were synthesized by an improved Suzuki cross-coupling method and their 13-*cis*-retinoic acid analogues were prepared in moderate to good yields via the Mitsunobu reaction. Their structures were confirmed by IR, ^{1}H NMR, ^{13}CNMR, MS spectra and element analysis and their antiproliferative activities were determined in vitro using human cancer cell lines. The results of bioassay showed that these organometallic analogues exhibited higher antiproliferative activities than parent 13-*cis*-retinoic acid and other retinoyl derivatives.

1. Introduction

Retinoids include active metabolites of vitamin A (retinol, **1**) as well as a diverse spectrum of natural or synthetic derivatives. They play an essential role in vertebrate growth and development, supporting cell differentiation, embryonic development, the immune response, and reproduction [1–3]. All-trans-retinoic acid (**2**, ATRA), 13-*cis*-retinoic acid (**3**, 13-*cis*-RA), and other retinoids are currently used for treatment of dermatological disorders and as chemotherapeutic agent against various endothelial cancers, breast cancer, and endometrial cancer [4,5]. The actions of retinoids are mediated through binding and activation of the retinoic acid receptors (RARs) or retinoid X receptors (RXRs), which function as ligand-dependent transcription factors [6]. However, retinoids, including retinoic acids, have been found to be too toxic at high dosage levels to be of practical value for cancer prevention in higher mammals. Side effects such as teratogenicity, hepatotoxicity, and headaches have been observed as a result when the most of these compounds were used [7]. Researchers have been pursuing the discovery and synthesis of novel retinoic acid analog in order to increase their therapeutic efficiency and/or reduced toxicity (Scheme 1) [8].

Recently, increasing interests have been focused on developing structural variations of established drugs by metallocenic organometallic compounds as alternatives of the chemotherapy of drug-resistance in cancer and tropical diseases [9–11]. The stability, non-toxicity and readily membrane-permeation of the ferrocenyl group, the accessibility of a large variety of derivatives, as well as its favourable electrochemical properties have made ferrocene and its derivatives very suitable for biological applications and for conjugation with biomolecules [12,13]. Several structural modification of established drugs with ferrocenyl moiety have been reported, such as ferrocene fluconazole [14], ferrocene aspirin [15], the anti-malarial drugs chloroquine (termed ferroquine), quinine, mefloquine, and artemisinin [16,17], and the anti-cancer drug tamixofen (termed ferrocifen) [18]. Accordingly, using ferrocenyl-containing derivatives as medicines and other chemotherapeutants has long been recognized as an attractive way.

In order to search novel retinoyl derivatives with potent antiproliferative activities, our research group had synthesized some sugar derivatives with structures of the glycosyl moiety both directly and indirectly linked with 13-*cis*-RA and obtained some biological compounds [19]. The results of biological assays showed that some compounds possessed some degree of antitumor activities. Following our continual interest in search of novel analogues of retinoic acid with potent biological activities, we have sought to design and synthesize a class of novel retinoates structurally modified by ferrocenyl group. In the present work, on the basis of an improved synthetic method of monoarylferrocenyl alcohols **9a** and **9b**, we herein reported their chemical and biological screening results involving their in vitro antitumor activities.

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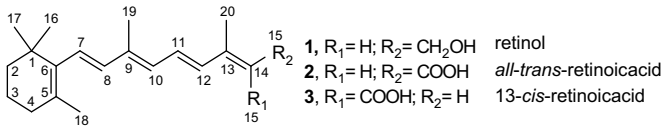


Scheme 1. Structures of retinol, all-trans-retinoic acid and 13-*cis*-RA.

2. Chemistry

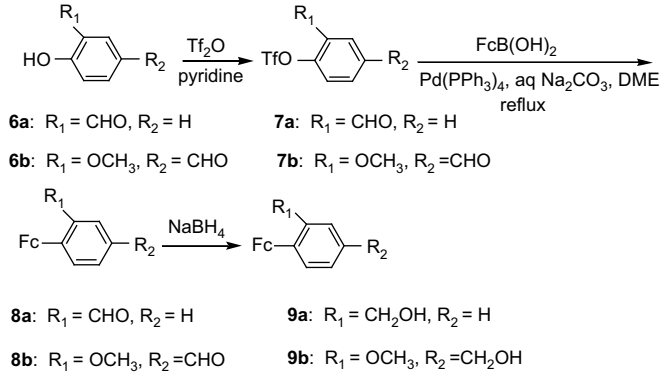
2.1. Synthesis of ferrocenyl alcohols *4a–4e* and ferrocenyl phenol *4f*

Ferrocenemethanol **4a** [20], Ferroceneethanol **4b** [20], Ferrocene propanol **4c** [20], Ferrocenebutanol **4d** [20], p-Ferrocenebenzyl alcohol **4e** [21] were prepared via well established routes. p-ferrocenyl phenol **4f** was prepared via reaction of diazotized 4-amino phenol with ferrocene according to a literature procedure [22].

2.2. Synthesis of monoarylferrocenyl alcohols *9a* and *9b*

The synthetic process of monoarylferrocenyl alcohols **9a** and **9b** were performed as shown in Scheme 2. The most frequently used method is an arylation process involving the reaction of ferrocene with aryldiazonium salts. However, the yields of monoarylferrocenes obtained in these reactions were lower than 40% in many cases [23]. Furthermore, these reactions are not specific and produce 1, 1′-, 1, 2- and 1, 3-diarylferrocenes which are normally difficult to separate. Transition metal-catalysed cross-coupling reactions have proved their value in synthetic and materials chemistry as tools for C–C bond formation and may be applied to the synthesis of diaryls containing virtually any functional group [24]. Introduction of the side chain has efficiently been accomplished by an improved Suzuki cross-coupling reaction between ferroceneboronic acid and aromatic triflates. In an initial experiment, the coupling between **7a** and ferroceneboronic acid gave only 20% of the coupling product under conventional Suzuki conditions [$Pd(PPh_3)_4$, K_2HPO_4, DMF, THF, 80 °C]. A systematic study of various solvents, bases, and catalysts revealed that the combination of $Pd(PPh_3)_4$ (0.05 equiv) and K_3PO_4 (2 equiv) in refluxing dioxane gave reproducibly high yields of the coupling reported product **8a** [25] and previously unreported compound **8b** under mild conditions.

The variables in the Suzuki reaction as applied to the synthesis of arylferrocenes were numerous. With this method, the byproduct boric acid was easily removed by aqueous extraction and posed no toxicological concern. This modification of the Suzuki cross-coupling reaction proved to be a clean and useful method for the preparation of monosubstituted arylferrocenes. For the present synthesis, we sought to apply the Suzuki Miyaura cross-coupling method to this more complex side chain. The details of this study will be disclosed at a later date.



Scheme 2. The synthetic route to monoarylferrocenyl alcohols **9a** and **9b**.

The reduction of the carbonyl group of **8a** was facile with sodium borohydride to give **9a**. The physical and spectral characteristics of **9a** were identical to the reported literature [25]. The unreported compound **9b** was prepared according to the similar procedure for **9a**, whose structure had been confirmed by IR, ^1H NMR and MS spectra.

2.3. Synthesis of ferrocenyl retinoates *5a–5h*

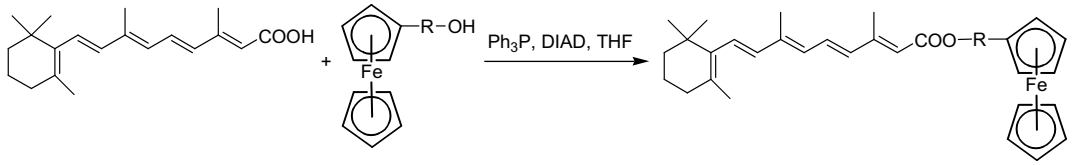
The esterification of 13-*cis*-retinoic acid had been reported in several ways. The reported reaction of 13-*cis*-RA to react with thionyl chloride (PCl_5/PCl_3 or other carbonyl chlorides) to form retinoyl chloride, which in turn is reacted with alcohols to produce retinoates, failed in our laboratory because hydrogen chloride in solution of retinoyl chloride isomerizes the polyene chain [26]. Initially we envisioned a carbodiimide-mediated coupling using DCC in combination with a catalytic amount of DMAP. Surprisingly, the reaction proved to be problematic, providing the desired retinoates in less than 60% yield after a prolonged reaction time (48 h) and use of a large excess (5 equiv) of DCC. Unfortunately, our attempts to optimize the conditions did not result in a significant enhancement of the reaction rate or yield. Finally we were intrigued by the possible application of the Mitsunobu reaction to construct our desired C–O bond and furnish retinoates. The Mitsunobu reaction, discovered in 1967 [27], is a robust and invaluable synthetic transformation that allows for the stereo-selective incorporation of azides [28], esters [29], nitriles [30], phthalimides [31], and sulfonamides [32] with inversion of configuration. Moreover, various protocols based on the Mitsunobu reaction have been developed for the coupling of alkyl alcohols with phenols or carboxylic acids as substrates. Many of these procedures have been modified for use on solid-phase supports [33]. As such, we envisioned the Mitsunobu reaction as an attractive route to pursue for the synthesis of retinoates. Gratifyingly, the experiment results proved that Mitsunobu reaction was a convenient and effective method for the esterification of 13-*cis*-RA with various ferrocenyl alcohols producing the corresponding retinoates in good to excellent yields (as shown in Table 1). It presents the advantages of mild conditions, high yield, and ease of operation.

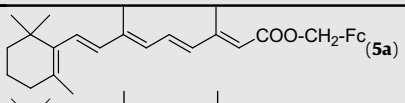
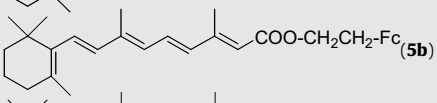
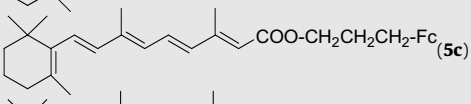
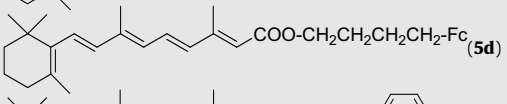
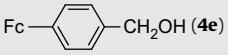
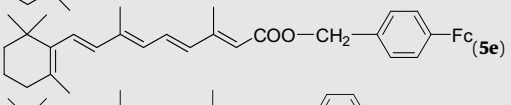
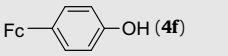
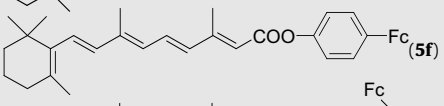
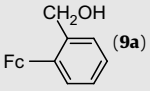
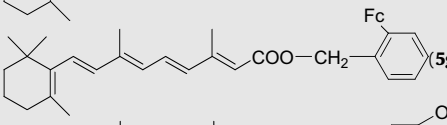
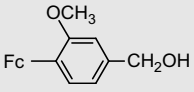
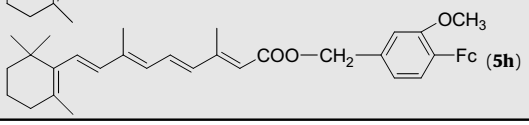
3. Biological studies

All 13-*cis*-Retinoyl ferrocene derivatives **5a–5h** were tested for antiproliferative activities toward three different cancer cell lines: human lung cancer cell line (A549), human liver cancer cell line (BEL7404), and human tongue cancer cell line (Tca). The results, expressed as the concentration of the compound required to kill the tumor cell by 50% (IC_{50}), are listed in Table 2. 13-*cis*-RA was used as a reference substance.

The screening data revealed that all selected compounds showed some antitumor activities, and were stronger than parent 13-*cis*-retinoic acid. It should be noted that the activity of the ferrocenyl retinoates **5a–5d** decreased with increasing of the ferrocenyl carbon chain. It is interesting to note that the other ferrocenyl retinoates **5e–5h** which include aromatic group showed some degree of antiproliferative activities, but exhibited lower activities than those with saturated straight-chain. Compared with our reported retinoyl sugar derivatives [19], the biological activities of this type of compounds are increased. It is interesting to note that the activities in vitro of the title compounds appeared to be weakly associated with the substituent group on the benzene cycle. The

Table 1
The Mitsunobu reaction of 13-*cis*-retinoic acid with ferrocenyl alcohols.



Entry	Fc–R–OH	Time (h)	Product	Yield (%)
1	$FcCH_2OH$ (**4a**)	2	 (**5a**)	83
2	$FcCH_2CH_2OH$ (**4b**)	2.5	 (**5b**)	82.5
3	$FcCH_2CH_2CH_2OH$ (**4c**)	2	 (**5c**)	82
4	$FcCH_2CH_2CH_2CH_2OH$ (**4d**)	2	 (**5d**)	81
5	 (**4e**)	2	 (**5e**)	80
6	 (**4f**)	3	 (**5f**)	75
7	 (**9a**)	3	 (**5g**)	76
8	 (**9b**)	2	 (**5h**)	78

derivative **5h** with electron-donating groups on phenyl fragment shows weaker antitumor activities, but the effect is not obvious.

4. Conclusion

Eight 13-*cis*-RA analogues were synthesized by structural modifications with ferrocenyl groups in order to decrease toxicities of 13-*cis*-RA and enhance its antiproliferative activities. Secondly, a new retinoylation method using the Mitsunobu reaction was developed after evaluating various reaction conditions. All selected compounds (**5a**–**5h**) were tested by MTT assay on growth of three different tumor cell lines. The results revealed that these compounds showed strong potential antitumor activities

5. Experimental protocols

5.1. General

13-*cis*-RA was purchased from Sigma Chemical Co. Ph_3P and diisopropyl azodicarboxylate (DIAD) were purchased from Alfa Aesar. The ferrocene and all other reagents were of the highest commercially available quality. All retinoids were stored at −20 °C under dry nitrogen atmosphere. All operations involved in the preparation, isolation, purification, and transfer of retinoids were carried out under an atmosphere of dry nitrogen. All operations were also performed in dim light or photographic darkroom light with containers wrapped with aluminum foil or black cloth. All reactions were monitored by TLC. Melting points were measured with a XRC-1 melting point apparatus and are uncorrected. FT infrared (IR) spectra were recorded in KBr disks using FD-5DX spectrometer. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Varian INOVA-400 Spectrometer, using $CDCl_3$ as a solvent; TMS (δ 0.00 ppm) was used as an internal standard. All NMR chemical shifts are reported as *d* values in parts per million (ppm) and coupling constants (*J*) are given in hertz (Hz). The splitting pattern abbreviations are as follows: s, singlet; d, doublet; br, broad peak; and m, multiplet. Mass spectra were carried out on a ZAB-HS mass spectrometer.

5.2. General procedure for the cross-couplings of ferroceneboronic acid with aryl triflates (*8a* and *8b*)

A mixture of ferroceneboronic acid (5.5 mmol), aryltriflate (5 mmol), $Pd(PPh_3)_4$ (0.25 mmol), and K_3PO_4 (10 mmol) in freshly distilled dioxane (30 mL) was heated to reflux under N_2 until no starting material could be detected by TLC. The product was extracted with ethyl acetate, washed with brine, and dried over $MgSO_4$. Isolation by column chromatography over silica gel gave following compounds **8a** and **8b**.

8a: yield 88%; yellow solid; m.p. 59–60 °C IR (KBr): 1684, 1589, 1495, 1259, 1194, 1105, 1010, 803, 689 cm^{-1}. ^{1}H NMR ($CDCl_3$): 3.50 (s, 5 H, Cp); 3.76 (t, 2 H, $J = 1.8$, C_5H_4-*m*); 3.89 (t, 2 H, $J = 1.8$, C_5H_4-*o*); 6.6–7.3 (m, Ar); 9.80 (s, 1 H, CHO).

8b: yield 85%; yellow solid; m.p. 82–83 °C; IR (KBr) 1689, 1593, 1513, 1266, 813, 704 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 3.97 (s, 3H,

Table 2
Cytotoxicity of ferrocenyl retinoates **5a**–**5h** against A549, BEL7404 and Tca cancer cell.

Compound	Cell line/IC_{50} (μM)		
	A549	BEL7404	Tca
5a	20.4	22.3	18.6
5b	21.5	24.9	19.7
5c	24.1	25.2	20.3
5d	26.0	27.6	21.7
5e	28.8	30.5	28.0
5f	29.3	31.1	30.1
5g	30.7	33.0	31.8
5h	31.2	37.2	32.5
13-*cis*-RA	35.8	42.6	39.7

OCH_3); 4.10 (s, 5H, Cp); 4.44 (s, 2H, C_5H_4-*m*); 4.93 (s, 2H, C_5H_4-*o*); 7.39 (s, 1H, Ar); 7.40 (s, 1H, Ar); 7.65 (d, 1H, Ar, $J = 17.6$ Hz); 9.95 (s, 1H, CHO).

5.3. A general synthetic method for compounds **9a** and **9b**

Compound **8** (4.0 mmol) was suspended methanol (100 mL) at room temperature with stirring. $NaBH_4$ (0.15 g, 4 mmol) was added with magnetic stirring. The reaction mixture was stirred for an additional hour at rt and then poured into ice-water (100 mL). The pH was adjusted to about 6.0–7.0. The mixture was extracted with ethyl acetate (150 mL × 3). The combined organic layers were washed with water (100 mL × 2), dried with $MgSO_4$, and evaporated by vacuum. The residue was isolated by column chromatography and gave following compounds **9a** and **9b**.

9a: yield: 91%; yellow oil. IR (KBr): 3094, 1634, 1385, 1107, 1032, 1005, 820, 764, 490 cm^{-1}. ^1H NMR ($CDCl_3$): 3.0 (bs, OH); 4.05(s, 5 H, Cp); 4.20(t, 2H, $J = 1.8$); 4.44 (t, 2 H, $J = 1.8$); 4.60 (s, 2 H); 7.16–7.28 (m, 4 H, Ar). ^{13}C NMR ($CDCl_3$): 64.2, 68.8, 69.0, 70.2, 70.5, 127.2, 128.7, 129.3, 129.7, 131.2, 131.6.

9b: yield: 90%; yellow solid. m.p. 125 °C. ^1H NMR (400 MHz, $CDCl_3$) δ: 3.76 (s, 3H, OCH_3); 4.44 (s, 5H, C_5H_5); 4.52 (s, 2H, C_5H_4-*m*); 4.90 (s, 2H, C_5H_4-*o*); 7.24–7.31(m, 3 H, Ar). IR (KBr) 3096, 1641, 1390, 1121, 1029, 500 cm^{-1}. MS: $m/z = 322$ ($[M + H]^+$).

5.4. General procedure for the synthesis of **5a**–**5h**

To a stirred solution of the ferrocenyl alcohol or phenol (3 mmol), PPh_3 (1.18 g, 4.5 mmol), and 13-*cis*-RA (3 mmol) in dry THF (20 mL) was added to DIAD (0.8 g, 4.5 mmol) under N_2 atmosphere at 0 °C. The reaction mixture was allowed to warm to rt and was stirred for additional 2 h. The reaction was monitored by TLC. The yellow reaction mixture was concentrated on a rotary evaporator (30 °C) to give a viscous oil. The residue was purified by column chromatography (ethyl acetate/petroleum ether, v/v) and gave retinoates **5a**–**5h** in various yields.

5a: yield: 83 %; orange yellow solid; m.p. 105–106 °C. Anal. calc. for $C_{31}H_{38}FeO_2$: C, 74.69; H, 7.68; found C, 74.76; H, 7.58; ^1H NMR (400 MHz, d_6-DMSO) δ: 1.03 (s, 6H, $2CH_3$); 1.30–1.50 (m, 2H, H on C_2); 1.60 (m, 2H, H on C_3); 1.71 (s, 3H, CH_3 on C_5); 1.99 (s, 3H, CH_3 on C_9); 2.00–2.05 (m, 2H, H on C_4); 2.06 (s, 3H, CH_3 on C_{13}); 4.17 (s, 5H, Cp); 4.21 (s, 2H, OCH_2); 4.30 (s, 2H, C_5H_4-*m*); 4.93 (s, 2H, C_5H_4-*o*); 5.62 (s, 1H, H on C_{14}); 6.15 (d, 1H, H_8, $J = 16.0$ Hz); 6.24 (d, 1H, H_{10}, $J = 11.2$ Hz); 6.27 (d, 1H, H_7, $J = 10.4$ Hz); 6.99 (dd, 1H, H_{11}, $J = 11.6$ Hz, $J = 11.6$ Hz); 7.79 (d, 1H, H_{12}, $J = 15.2$ Hz). ^{13}C NMR (400 MHz, d_6-DMSO) δ 12.9, 19.3, 21.0, 21.8, 29.0, 33.1, 34.3, 39.7, 62.0, 68.6, 68.7, 69.5, 76.7, 77.0, 77.3, 116.4, 128.5, 129.4, 130.0, 130.4, 132.2, 137.5, 137.7, 139.8, 151.4, 166.1. IR (KBr) 2924, 1707, 1232, 1140, 974, 833, 488 cm^{-1}. MS: $m/z = 499$ ($[M + H]^+$)

5b: yield: 82.5 %; orange yellow solid; m.p. 102–103 °C. Anal. calc. for $C_{32}H_{40}FeO_2$: C, 74.99; H, 7.87, found C, 75.11; H, 7.79. ^1H NMR (400 MHz, d_6-DMSO) δ: 1.02 (s, 6H, $2CH_3$); 1.44–1.45 (m, 2H, H on C_2); 1.58 (m, 2H, H on C_3); 1.70 (s, 3H, CH_3 on C_5); 1.97 (s, 3H, CH_3 on C_9); 2.00–2.05 (m, 2H, H on C_4); 2.08 (s, 3H, CH_3 on C_{13}); 2.62 (t, 2H, $J = 6.8$ Hz, CH_2CH_2); 4.08 (s, 5H, Cp); 4.14 (t, 2H, $J = 14.4$ Hz, OCH_2CH_2); 4.19 (s, 2H, C_5H_4-*m*); 4.21 (s, 2H, C_5H_4-*o*); 5.70 (s, 1H, H on C_{14}); 6.22 (d, 1H, H_8, $J = 13.6$ Hz); 6.25 (d, 1H, H_{10}, $J = 11.2$ Hz); 6.28 (d, 1H, H_7, $J = 10.4$ Hz); 7.08 (dd, 1H, H_{11}, $J = 11.6$ Hz, $J = 15.2$ Hz); 7.68 (d, 1H, H_{12}, $J = 15.2$ Hz). ^{13}C NMR (400 MHz, d_6-DMSO) δ 12.9, 19.3, 21.0, 21.8, 29.0, 33.1, 34.3, 39.7, 62.0, 68.6, 68.7, 69.5, 76.7, 77.0, 77.3, 116.4, 128.5, 129.4, 130.0, 130.4, 132.2, 137.5, 137.7, 139.8, 151.4, 166.1. IR (KBr) 2930, 1715, 1232, 1140, 974, 492 cm^{-1}. MS: $m/z = 513$ ($[M + H]^+$)

5c: yield: 82 %; orange yellow oil. Anal. calc. for $C_{33}H_{42}FeO_2$: C, 75.28; H, 8.04; found C, 75.36; H, 8.00; ^1H NMR (400 MHz, d_6-DMSO) δ: 1.02 (s, 6H, $2CH_3$); 1.43–1.46 (m, 2H, H on C_2); 1.56–1.61 (m, 2H, H on C_3); 1.69 (s, 3H, CH_3 on C_5); 1.81 (m, 2H, CH_2); 2.00 (s, 3H, CH_3 on C_9); 2.01–2.03 (m, 2H, H on C_4); 2.08 (s, 3H, CH_3 on C_{13}); 2.35 (t, 2H, $J = 7.6$ Hz, CH_2); 4.05 (s, 5H, Cp); 4.08 (t, 2H, $J = 6.8$ Hz, OCH_2CH_2); 4.12 (s, 2H, C_5H_4-*m*); 4.20 (s, 2H, C_5H_4-*o*); 5.72 (s, 1H, H on C_{14}); 6.22 (d, 1H, H_8, $J = 16$ Hz); 6.28 (d, 1H, H_{10}, $J = 11.2$ Hz); 6.31 (d, 1H, H_7, $J = 6.8$ Hz); 7.07 (dd, 1H, H_{11}, $J = 11.6$ Hz, $J = 11.6$ Hz); 7.68 (d, 1H, H_{12}, $J = 15.6$ Hz). ^{13}C NMR (400 MHz, d_6-DMSO) δ 12.9, 19.3, 21.0, 21.8, 29.0, 33.1, 34.3, 39.7, 62.0, 68.6, 68.7, 69.5, 76.7, 77.0, 77.3, 116.4, 128.5, 129.4, 130.0, 130.4, 132.2, 137.5, 137.7, 139.8, 151.4, 166.1. IR (KBr) 2929, 1730, 1242, 1136, 974, 496 cm^{-1}. MS: $m/z = 527$ ($[M + H]^+$)

5d: yield: 81 %; orange yellow solid; m.p. 61–62 °C. Anal. calc. for $C_{34}H_{44}FeO_2$: C, 75.55; H, 8.20; found C, 75.62; H, 8.14; ^1H NMR (400 MHz, d_6-DMSO) δ: 1.09 (s, 6H, $2CH_3$); 1.44–1.45 (m, 2H, H on C_2); 1.56–1.61 (m, 2H, H on C_3); 1.45–1.60 (m, 4H, CH_2CH_2); 1.62 (s, 3H, CH_3 on C_5); 1.81 (m, 2H, CH_2); 2.00 (s, 3H, CH_3 on C_9); 2.01–2.03 (m, 2H, H on C_4); 2.08 (s, 3H, CH_3 on C_{13}); 2.40 (t, 2H, $J = 7.6$ Hz, CH_2); 4.03 (s, 5H, Cp); 4.05 (t, 2H, $J = 6.8$ Hz, OCH_2CH_2); 4.10 (s, 2H, C_5H_4-*m*); 4.14 (s, 2H, C_5H_4-*o*); 5.69 (s, 1H, H on C_{14}); 6.21 (d, 1H, H_8, $J = 16.4$ Hz); 6.25 (d, 1H, H_{10}, $J = 4.8$ Hz); 6.29 (d, 1H, H_7, $J = 15.6$ Hz); 7.06 (dd, 1H, H_{11}, $J = 12$ Hz, $J = 11.2$ Hz); 7.67 (d, 1H, H_{12}, $J = 15.2$ Hz). ^{13}C NMR (400 MHz, d_6-DMSO) δ 12.9, 19.0, 20.8, 21.8, 27.3, 28.4, 28.8, 29.1, 32.9, 34.1, 39.2, 39.4, 39.6, 39.8, 40.0, 40.2, 40.4, 63.4, 67.1, 68.0, 68.6, 88.8, 116.5, 128.4, 129.3, 129.9, 130.6, 132.9, 137.3, 137.5, 140.3, 151.5, 165.9. IR (KBr) 2929, 1730, 1242, 1136, 974, 496 cm^{-1}. MS: $m/z = 541$ ($[M + H]^+$)

5e: yield: 80 %; orange yellow solid; m.p. 128–129 °C. Anal. calc. for $C_{37}H_{42}FeO_2$: C, 77.34; H, 7.37; found C, 77.41; H, 7.25; ^1H NMR (400 MHz, d_6-DMSO) δ: 1.03 (s, 6H, $2CH_3$); 1.44–1.45 (m, 2H, H on C_2); 1.56–1.61 (m, 2H, H on C_3); 1.62 (s, 3H, CH_3 on C_5); 2.00 (s, 3H, CH_3 on C_9); 2.01–2.03 (m, 2H, H on C_4); 2.08 (s, 3H, CH_3 on C_{13}); 4.05 (s, 5H, Cp); 4.06 (s, 2H, OCH_2); 4.32 (s, 2H, C_5H_4-*m*); 4.66 (s, 2H, C_5H_4-*o*); 5.70 (s, 1H, H on C_{14}); 6.21 (d, 1H, H_8, $J = 16.4$ Hz); 6.25 (d, 1H, H_{10}, $J = 4.8$ Hz); 6.29 (d, 1H, H_7, $J = 15.6$ Hz); 7.06 (dd, 1H, H_{11}, $J = 12$ Hz, $J = 11.2$ Hz); 7.46 (s, 2H, C_6H_4); 7.29(s, 2H, C_6H_4); 7.82 (d, 1H, H_{12}, $J = 15.6$ Hz). ^{13}C NMR (400 MHz, d_6-DMSO) δ 12.9, 19.2, 21.0, 21.8, 28.9, 29.0, 33.1, 34.3, 39.6, 65.5, 66.8, 69.3, 69.9, 76.71, 77.0, 77.3, 116.2, 126.3, 128.3, 128.6, 129.3, 130.1, 130.3, 132.4, 137.5, 137.7, 139.3, 151.7. IR (KBr) 2929, 1730, 1242, 1136, 974, 496 cm^{-1}. MS: $m/z = 574$ ($[M + H]^+$)

5f: yield: 75 %; orange yellow solid; m.p. 137–138 °C. Anal. calc. for $C_{36}H_{40}FeO_2$: C, 77.14; H, 7.19; found C, 77.26; H, 7.10; ^1H NMR (400 MHz, d_6-DMSO) δ: 1.02 (s, 6H, $2CH_3$); 1.45–1.47 (m, 2H, H on C_2); 1.60–1.61 (m, 2H, H on C_3); 1.71 (s, 3H, CH_3 on C_5); 2.01 (s, 3H, CH_3 on C_9); 2.02–2.03 (m, 2H, H on C_4); 2.17 (s, 3H, CH_3 on C_{13}); 4.05 (s, 5H, Cp); 4.31 (s, 2H, C_5H_4-*m*); 4.61 (s, 2H, C_5H_4-*o*); 5.87 (s, 1H, H on C_{14}); 6.13 (d, 1H, H_8, $J = 16$ Hz); 6.23 (d, 1H, H_{10}, $J = 4.8$ Hz); 6.28 (d, 1H, H_7, $J = 15.6$ Hz); 7.03 (dd, 1H, H_{11}, $J = 12$ Hz, $J = 11.2$ Hz); 7.06

(s, 2H, C_6H_4); 7.48 (s, 2H, C_6H_4); 7.84 (d, 1H, H_{12}, $J = 15.2$ Hz). ^{13}C NMR (400 MHz, d_6-DMSO) δ 12.9, 19.2, 21.2, 21.7, 29.0, 33.1, 34.3, 39.6, 66.9, 69.5, 70.2, 76.7, 77.0, 77.2, 77.3, 115.2, 121.3, 121.5, 127.0, 129.0, 129.1, 130.2, 133.2, 136.5, 137.4, 137.7, 140.5, 149.1, 153.8, 164.7. IR (KBr) 2925, 1724, 1207, 1120, 960, 818, 503 cm^{-1}. MS: $m/z = 560$ ($[M + H]^+$)

5g: yield: 76 %; orange yellow oil. Anal. calc. for $C_{37}H_{42}FeO_2$: C, 77.34; H, 7.37; found C, 77.39; H, 7.31; ^1H NMR (400 MHz, d_6-DMSO) δ: 1.02 (s, 6H, $2CH_3$); 1.44–1.45 (m, 2H, H on C_2); 1.56–1.61 (m, 2H, H on C_3); 1.62 (s, 3H, CH_3 on C_5); 2.00 (s, 3H, CH_3 on C_9); 2.01–2.03 (m, 2H, H on C_4); 2.08 (s, 3H, CH_3 on C_{13}); 4.15 (s, 5H, Cp); 4.21 (s, 2H, OCH_2); 4.31 (s, 2H, C_5H_4-*m*); 4.51 (s, 2H, C_5H_4-*o*); 5.72 (s, 1H, H on C_{14}); 6.21 (d, 1H, H_8, $J = 16.4$ Hz); 6.25 (d, 1H, H_{10}, $J = 4.8$ Hz); 6.28 (d, 1H, H_7, $J = 15.6$ Hz); 7.06 (dd, 1H, H_{11}, $J = 12$ Hz, $J = 11.2$ Hz); 7.25 (s, 2H, C_6H_4); 7.41 (s, 2H, C_6H_4); 7.82 (d, 1H, H_{12}, $J = 15.6$ Hz). ^{13}C NMR (400 MHz, d_6-DMSO) δ 12.8, 19.1, 21.2, 21.9, 29.1, 29.3, 33.1, 34.6, 39.8, 65.7, 66.9, 69.6, 67.0, 76.9, 77.1, 77.3, 116.9, 126.8, 128.3, 128.1, 129.9, 130.7, 130.5, 132.6, 137.9, 137.1, 139.9, 152.3. IR (KBr) 2929, 1730, 1242, 1136, 974, 496 cm^{-1}. MS: $m/z = 574$ ($[M + H]^+$)

5h: yield: 78 %; orange yellow oil. Anal. calc. for $C_{38}H_{44}FeO_3$: C, 75.49; H, 7.34; found C, 75.42; H, 7.33; ^1H NMR (400 MHz, d_6-DMSO) δ: 1.01 (s, 6H, $2CH_3$); 1.42–1.45 (m, 2H, H on C_2); 1.56–1.58 (m, 2H, H on C_3); 1.69 (s, 3H, CH_3 on C_5); 2.00 (s, 3H, CH_3 on C_9); 2.01–2.03 (m, 2H, H on C_4); 2.08 (s, 3H, CH_3 on C_{13}); 3.86 (s, 3H, OCH_3); 4.00 (s, 5H, Cp); 4.28 (s, 2H, C_5H_4-*m*); 4.76 (s, 2H, C_5H_4-*o*); 5.10 (s, 2H, OCH_2); 5.77 (s, 1H, H on C_{14}); 6.20 (d, 1H, H_8, $J = 16.4$ Hz); 6.23 (d, 1H, H_{10}, $J = 8.8$ Hz); 6.29 (d, 1H, H_7, $J = 16.0$ Hz); 6.91 (dd, 1H, H_{11}, $J = 12$ Hz, $J = 11.2$ Hz); 7.03 (s, 1H, C_6H_3); 7.25 (s, 1H, C_6H_3); 7.52 (d, 1H, C_6H_3, $J = 7.6$ Hz); 7.70 (d, 1H, H_{12}, $J = 15.2$ Hz). ^{13}C NMR (400 MHz, d_6-DMSO) δ 13.0, 19.0, 20.9, 21.8, 29.1, 32.9, 34.2, 39.2, 39.4, 39.5, 39.6, 39.8, 40.0, 40.2, 40.4, 55.7, 65.3, 68.5, 68.9, 69.5, 82.2, 11.6, 116.2, 120.5, 126.8, 128.6, 129.1, 129.3, 130.0, 130.5, 133.2, 135.6, 137.3, 137.5, 140.5, 152.1, 156.5, 165.7. IR (KBr) 2930, 1739, 1240, 1145, 970, 500 cm^{-1}. MS: $m/z = 594$ ($[M + H]^+$)

5.5. In vitro cytotoxicity assay

The antiproliferative activities of **5a**–**5h** were assessed by use of the MTT assay. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazol-ium bromide) assay is a simple nonradioactive colorimetric assay to measure cell cytotoxicity, proliferation, or viability. MTT is a yellow, water-soluble, tetrazolium salt. Metabolically active cells are able to convert this dye into a water-insoluble dark blue formazan by reductive cleavage of the tetrazolium ring. Formazan crystals then can be dissolved and quantified by measuring the absorbance of the solution at 570 nm, and the resultant value is related to the number of living cells.

The effect of **5a** on the cells' proliferation efficiency was determined after 24 h incubation with cells. To determine cell proliferation, the A549 cell lines, BEL7404 cell lines, and Tca cell lines were individually plated at a density of 1×10^4 cells/well in 96 well plates at 37 °C in 5% CO_2 atmosphere. After 24 h of culture, the medium in the wells was replaced with the fresh medium containing **5a** of varying concentrations respectively. The **5a** concentration given upon is the final concentration in the well. Every concentration added five wells as parallel control. After 24, 10 μL of MTT dye solution (5 mg/mL in phosphate buffer pH 7.4) was added to each well and incubated for 4 h at 37 °C and 5% CO_2 for exponentially growing cells and 10 min for steady-state confluent cells. The formazan crystals were solubilized with 100 μL of DMSO and the solution was vigorously mixed to dissolve the reacted dye. The absorbance of each well was read on a microplate reader (DYNATECH MR7000 instruments) at 570 nm. The spectrophotometer was calibrated to zero using culture medium without cells. We selected inhibitory effect to evaluate side effects of the silica-coated fluorescent 5**a** to cells proliferation. The inhibitory effect of **5a** was calculated as percentage inhibition in comparison to the value obtained in untreated well to which no **5a** was added. All the other compounds were determined by the same method.

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