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Remarkable anti-breast cancer activity of ferrocene tagged multi-functionalized 1,4-dihydropyrimidines

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ABSTRACT

A novel series of ferrocene tagged multi-functionalized 1,4-dihydropyrimidines is synthesized by base catalyzed cyclocondensation between ferrocenyl chalcones and amidines. The structures of synthesized compounds were established on the basis of ^{1}H NMR, ^{13}C NMR, FTIR spectroscopy as well as by mass spectrometry. The compounds were evaluated for *in vitro* anticancer activity. The most active compounds from the series displayed GI_{50} value equal to doxorubicin against the strain of human breast cancer cell line MDA-MB-435.

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1. Introduction

The advent of ferrocene through the serependitious discovery by Pauson and Kealy in 1951 has sparked off a significant inter-disciplinary research activity [1]. The unique structure with Fe^{+2} ion sandwiched between two pentahapto cyclopentadienyl ligands and three dimensional aromatic system has made ferrocene a versatile scaffold for covalent functionalization. Owing to its unique geometry, tunable redox properties and the high reactivity, ferrocenyl compounds find wide applications in diverse areas such as catalysis [2], materials science [3], analytical sensors [4], non linear optics [5], bio-organometallic and biological chemistry [6], organic synthesis [7] and in electrochemistry [8]. The relative stability of ferrocene in biological media and low levels of *in vivo* toxicity of its derivatives has spurred considerable interest in the development of ferrocene based compounds for cancer therapeutics. The inclusion of ferrocene in anticancer drug design strategies received a decisive impetus as Jaouen and co-workers [9] reported on ferrocenyl analogs of tamoxifen *viz* ferrocifen (**1**, Fig. 1) and hydroxyferrocifen (**2**, Fig. 1). *In vitro* cytotoxicity assays have revealed that ferrocifen is more effective than tamoxifen against both estrogen-dependent and -independent breast cancer. These findings have stimulated enormous interest in design and synthesis of new lead structures based on ferrocenyl moiety in drug discovery programs aimed at the development of potential anticancer agents [10]. It is believed that ferrocene tagged organic frameworks can provide new impetus for building structures with unprecedented attributes to probe and modulate anticancer activities.

The 1,4-dihydropyrimidine (1,4-DHPM) core is ubiquitous in both naturally occurring and synthetic biologically active molecules and many of its derivatives display interesting anti-viral, anti-tumor, anti-bacterial, and anti-inflammatory activities [11,12]. In addition, they are also regarded as calcium channel blockers [13], α-adrenergic antagonists as well as HIV gp-120-CD4 inhibitors [14]. Recently, two prototype members of 1,4-DHPM family *viz* Bay 41-4109 [methyl-4-(2-chloro-4-fluorophenyl)-2-(3,5-difluoro-2-pyridinyl)-6-methyl-1, 4-dihdro pyrimidine-5-carboxylate] (**3,** Fig. 1) and Bay 39-5493 [methyl-4-(2-chloro-4-fluorophenyl)-2-(-2-thiazyl)-6-methyl-1,4-di hdro-pyrimidine-5-carboxylate] (**4**, Fig. 1) have displayed prominent anti-hepatitis B replication activity [15]. Moreover, 1,4-DHPM derivatives such as **5** and **6** (Fig. 1) have been proved as selective ROCK1 inhibitors. ROCK1 is a potential therapeutic target in the treatment of cardiovascular diseases [16]. The anticancer potential of 1,4-DHPMs has been explored recently [17]. These compounds owe their anticancer activity to their ability to inhibit human kinesin Eg5 activity.

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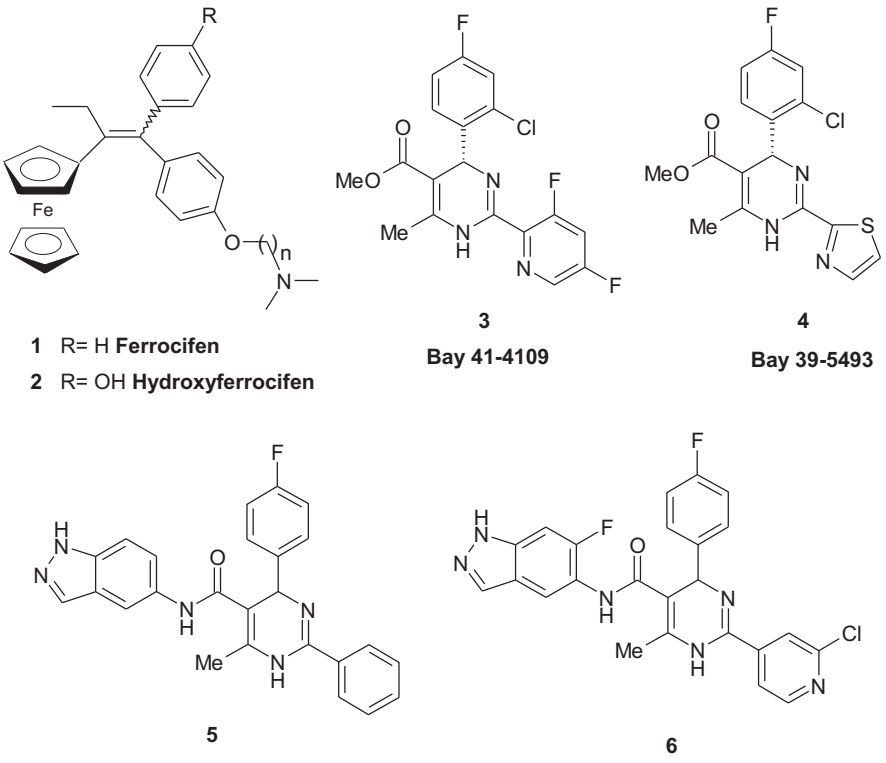


Fig. 1. Pharmacologically important ferrocene and 1,4-DHPM based compounds.

Human Eg5 has been an interesting drug target for the development of cancer chemotherapeutics as it plays a vital role in mitosis by establishing the bipolar spindle, the inhibition of which leads to mitotic arrest and cell death. Because of interesting biological properties and several degrees of structural diversity, development of novel methods for synthesis of 1,4-DHPMs as well as new analogs is an intensively investigated field of utmost importance.

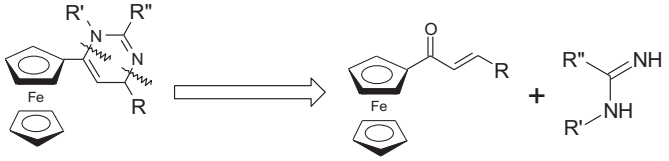
Based on these precedents, we sought to construct hybrid molecular architectures by combining ferrocene and pyrimidine pharmocophores. We envisioned that resultant conjugates by virtue of the presence of critical structural features might serve as a prototype for new drugs that could be used in anticancer research. In continuation of research work related to applications of ferrocene [18], we report herein synthesis and *in vitro* anti breast cancer activity of novel ferrocene tagged multi-functionalized 1,4-dihydropyrimidines.

2. Results and discussion

The initially planned retrosynthesis of ferrocene tagged multi-functionalized 1,4-DHPMs is depicted in Scheme 1 and is based on cyclocondensation between chalcones and amidine. This convergent route was envisaged to be highly significant due to easy accessibility of large number of structurally diverse ferrocenyl chalcones which can be easily prepared by reacting acetyl ferrocene with large variety of commercially available aryl aldehydes under basic conditions [19]. In addition, we hypothesized that use of substituted benzamidines that have already displayed promising biological activities [20] will offer a valuable addendum in biological profile.

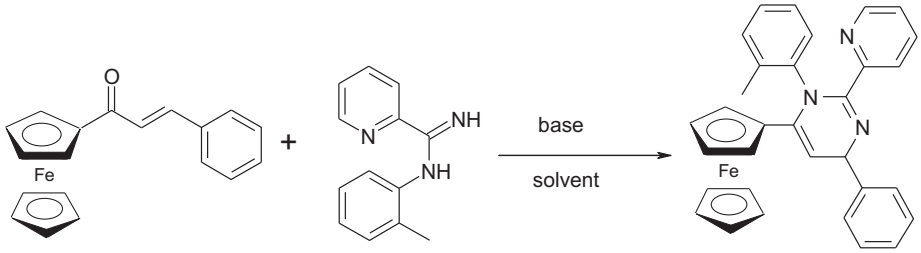
To test the validity of our disconnection approach, a preliminary survey of reaction conditions was conducted using 1-ferrrocenyl-3-phenyl-2-propen-1-one (a prototype ferrocenyl chalcone) and *N*-*o*-tolylpicolinamidine (Table 1). Our initial studies focused on scrutiny of bases required for cyclocondensation. A series of experiments were conducted using various bases in different solvents. The use of sodium metal in isopropanol was found to be most efficient catalytic system as it afforded the desired 6-ferrocenyl-4-phenyl-2-pyridin-2-yl-1-*o*-tolyl-1,4-dihydropyrimidine in 80% yield within 3 h under reflux conditions (Table 1, entry 1). The use of inorganic bases such as NaOH and KOH showed remarkably poor catalytic activity as the corresponding product was obtained in a scarce yield along with the formation of undesired tarry material after prolonged reaction time (Table 1, entries 2–5). On the other hand, the use of Na in ethanol furnished the anticipated product in moderate yield (Table 1, entry 5). Thus, use of sodium in IPA under reflux conditions was selected as optimal reaction conditions for further studies.

After the optimization of reaction conditions, we evaluated the scope of the protocol by reacting structurally diverse ferrocenyl chalcones with different amidines (Scheme 2). The results are summarized in Table 2. In general, the corresponding ferrocene tagged multi-functionalized 1,4-DHPMs were obtained in good to excellent yields in all the investigated cases. No significant electronic effects were observed for the electron donating as well as electron withdrawing substituents on phenyl ring in ferrocenyl chalcones. It was interesting to observe that Na/IPA catalytic system is tolerant to a halogen substitution on the phenyl ring thus providing a handle for additional synthetic manipulation *via* cross coupling chemistry.



Scheme 1. Retrosynthetic analysis of ferrocene tagged multi-functionalized 1,4-DHPMs.

Table 1
Optimization of reaction conditions.[a]



Entry	Base	Temp (°C)	Solvent	Time (h)	Yield[b] (%)
1	Na	80	Isopropanol	3	80
2	NaOH	80	Ethanol	10	30
3	NaOH	60	Methanol	7	25
4	KOH	80	Ethanol	10	35
5	KOH	80	DMF	10	40
6	Na	80	Ethanol	5	58

[a] All products were characterized by IR, ^{1}H and ^{13}C NMR spectroscopy as well as by mass spectrometry.
[b] Isolated yields after chromatography.

The structures of all the ferrocene tagged multi-functionalized 1,4-DHPMs were elucidated on the basis of ^{1}H NMR and ^{13}C NMR spectroscopy as well as by FTIR and mass spectrometry. The spectral data is in agreement with the proposed structures. All the synthesized compounds are air and moisture stable for several weeks without any decomposition and are soluble in normal organic solvents.

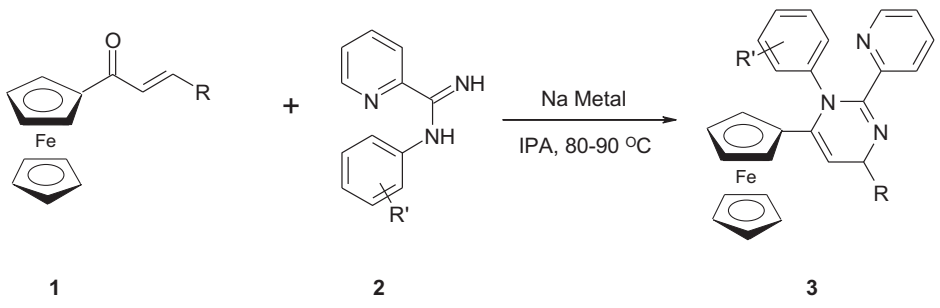
The UV–vis absorption spectra of all the synthesized compounds were measured in methanol (Fig. 2) and the optical characteristics are summarized in Table 3. The studies revealed that the absorption peaks of ferrocene tagged multi-functionalized 1,4-DHPMs were different from precursor ferrocenyl chalcones as the former display peaks in the range 270–318 nm due to π–π^* transitions of the ferrocene chromophore while latter exhibit the same in the range 487–517 nm [21]. The hypsochromic shift observed may be attributed to the disturbance in enone system of ferrocenyl chalcones during the cyclo-condensation with amidines. In addition, the less intensity absorption peaks in the region 450–550 nm were also observed. These peaks may be assigned to the metal to ligand charge transfer (MLCT) transitions from Fe to either antibonding or nonbonding orbitals of Cp rings.

A tentative mechanism for the formation of ferrocene tagged multi-functionalized 1,4-DHPMs is depicted in Scheme 3. The reaction is likely to proceed *via* sodium isopropoxide induced 1,4-nuclepohilic addition of amidine on ferrocenyl chalcone. An intramolecular displacement leads to nucleophilic addition furnishing cyclized intermediate. Under experimental reaction conditions, the cyclized intermediate loses water forming the final product.

All the synthesized ferrocene tagged multifunctionalized 1,4-DHPMs (**3a**–**j**) were evaluated for their *in vitro* cytotoxicity in human breast cancer cell line MDA-MB-435 by employing the sulforhodamine B (SRB) assay method [22]. The results are described in Table 4. It is worthy of note that all the compounds except **3h**–**j** were significantly cytotoxic against MDA-MB-435 compared to the standard drug tested, i.e. Doxorubicin, with the concentration of the drug that produced 50% inhibition of cell growth (GI_{50}) ranging from 17.4 to 152.9 μM. With regard to anticancer activity, **3b** and **3c** turned out to be the most attractive since they demonstrated the lowest GI_{50} values of 18 and 17.4 μM respectively in the cell line examined which was almost equal to the standard anticancer agent Doxorubicin (18.4 μM).

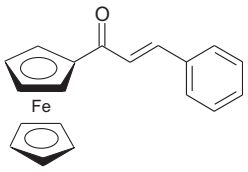
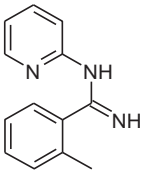
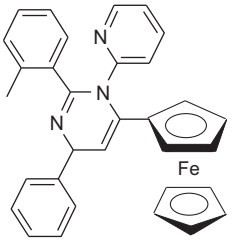
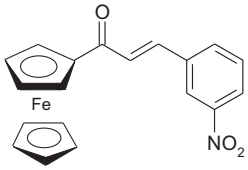
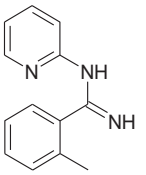
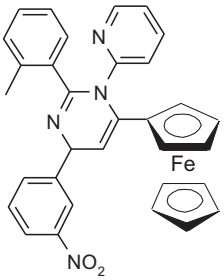
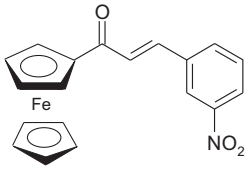
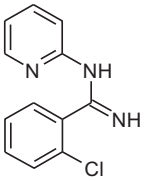
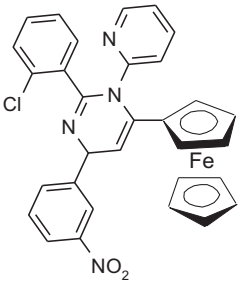
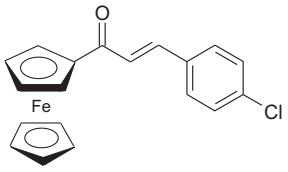
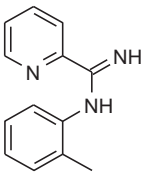
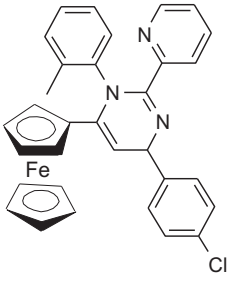
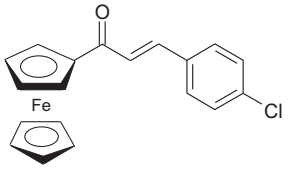
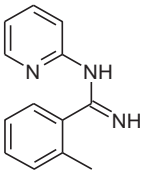
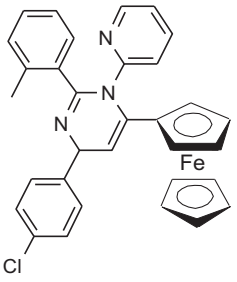
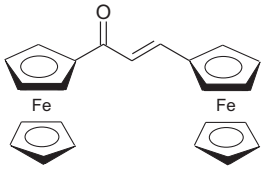
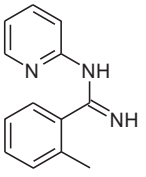
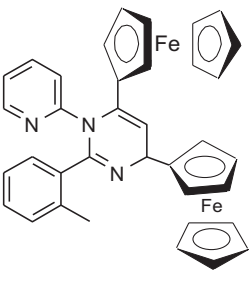
3. Conclusion

In summary, we have developed a reliable and generally applicable approach for the synthesis of a novel series of ferrocene tagged multi-functionalized 1,4-DHPMs from easily accessible ferrocenyl chalcones and amidines. The structures of all the compounds were established on the basis of analytical and spectral data. The synthesized compounds were evaluated for their *in vitro* anticancer activities against the strain of human breast cancer cell line MDA-MB-435. The compounds **3b** and **3c** with a GI_{50} values 18 and 17.4 μM respectively were found to be the most active. We believe that reported protocol offers scope for extension to variety of other substrates to form products that might serve as an interesting alternative in endemic area of anticancer research.



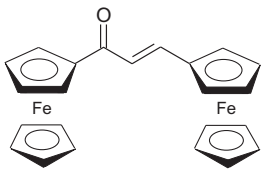
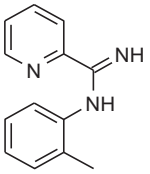
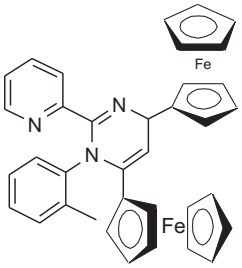
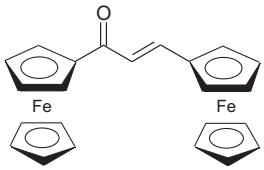
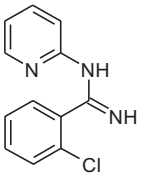
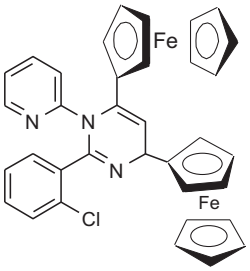
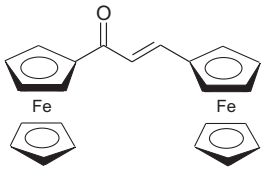
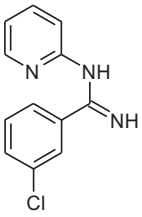
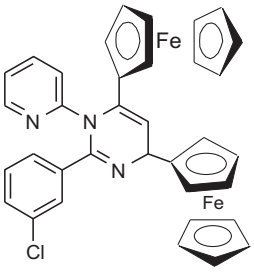
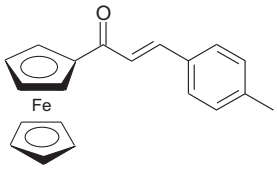
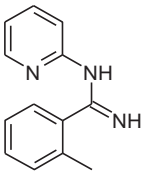
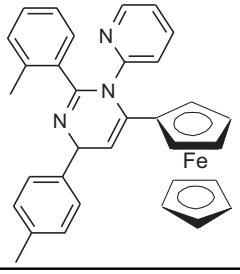
Scheme 2. Na/IPA catalyzed cyclocondensation of ferrocenyl chalcones with amidines.

Table 2
Synthesis of ferrocene tagged multi-functionalized 1,4-DHPMs.[a]

Entry	Ferrocenyl chalcone (**1**)	Amidine (**2**)	Product (**3**)	Yield[b] (%)
a				80
b				88
c				85
d				82
e				72
f				70

(*continued on next page*)

Table 2 (*continued*)

Entry	Ferrocenyl chalcone (**1**)	Amidine (**2**)	Product (**3**)	Yield[b] (%)
g				75
h				65
i				68
j				76

[a] All products were characterized by IR, ^{1}H and ^{13}C NMR spectroscopy as well as by mass spectrometry.
[b] Isolated yields after chromatography.

4. Experimental part

4.1. General

^{1}H NMR and ^{13}C NMR spectra were recorded on a Brucker AC (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR) spectrometer using $CDCl_3$ as solvent and tetramethylsilane (TMS) as an internal standard. Infrared spectra were recorded on a Perkin–Elmer FTIR spectrometer. The samples were examined as KBr discs ~5% w/w. Mass spectra were recorded on a Perkin Elmer Flexar SQ 300 LCMS. Elemental analyses were performed on EURO EA3000 vectro model. Melting points were determined on MEL-TEMP capillary melting point apparatus and are uncorrected. Precoated aluminum sheets (silicagel 60 F254, Merck Germany) were used for thin-layer chromatography (TLC) and spots were visualized under UV light. All the ferrocenyl chalcones [19] and benzamidines [20] were synthesized following the literature procedure. All other chemical were obtained from local chemical; suppliers and were used without further purification.

4.2. General procedure for the synthesis of ferrocene tagged multi-functionalized 1,4-dihydropyrimidines (*3a–j*)

A mixture of sodium metal (69 mg, 3 mmol) in isopropanol (25 ml) was refluxed for 30 min. Amidine (1 mmol) and ferrocenyl chalcone (1 mmol) were sequentially charged into reaction flask at intervals of 30 min. The reaction mixture was heated under reflux conditions for 3–5 h. After completion of the reaction as monitored by thin layer chromatography (TLC), the reaction mixture was poured into ice-water and extracted with ethyl acetate (3 × 25 ml). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to

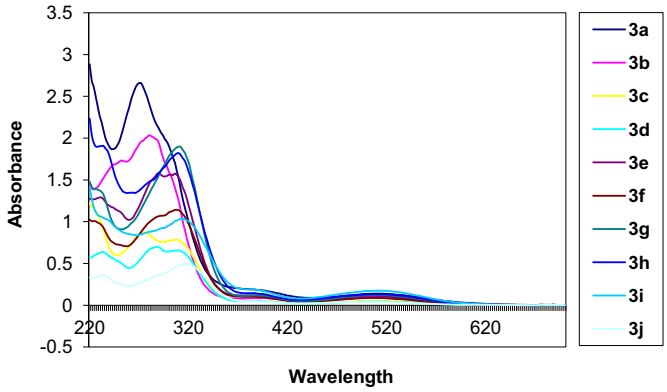


Fig. 2. UV–vis absorption spectra of 1,4 DHPMs in methanol.

give crude residue which was purified by column chromatography over silica gel using hexane/ethyl acetate (4:1 v/v) to afford the pure compound.

*4.2.1. 6-Ferrocenyl-4-phenyl-1-pyridin-2-yl-2-o-tolyl-1,4-dihydro-pyrimidine (**3a**)*

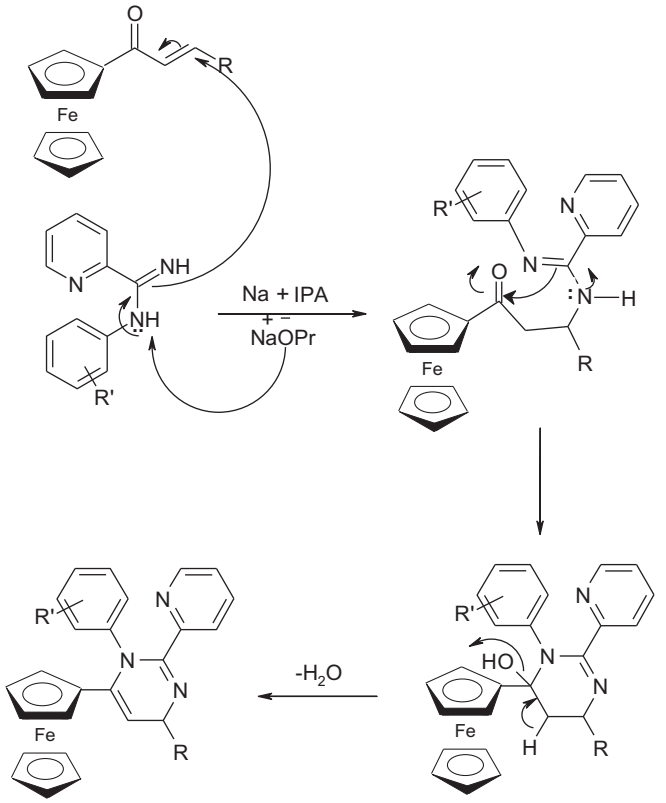
Reddish brown solid; m.p: 144–146 °C; Anal. calc. for $C_{32}H_{27}FeN_3$: C 75.44, H 5.34; N 8.24%. Found: C 75.56, H 5.77, N 8.93%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.6 (d, 2H, $J = 4$ Hz, Py-H), 7.91–7.84 (m, 4H, Ar–H), 7.79 (m, 3H, Ar–H), 7.67 (m, 2H, Ar–H), 7.28 (m, 3H, Ar–H), 7.13 (d, 1H, $J = 6.3$ Hz, Ar–H), 4.93 (s, 2H, ferrocene), 4.60 (s, 2H, ferrocene), 4.23 (s, 5H, ferrocene), 2.24 (s, 3H, methyl-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.0, 145.9, 139.4, 138.0, 135.9, 134.7, 133.7, 129.9, 129.4, 129.2, 127.9, 125.7, 123.4, 121.0, 119.1(Ar–C), 80.45, 72.9, 70.14, 69.7 (ferrocene-C), 15.3 (methyl-C); IR (KBr, thin film): $\upsilon = 3052$ (Ar–H), 2951 (C–H), 1532 (C=N), 1456 (C=C), 1244 (C–N); MS (EI): $m/z = 509$.

*4.2.2. 4-(3-Nitro-phenyl)-6-ferrocenyl-1-pyridin-2-yl-2-o-tolyl-1,4-dihydro-pyrimidine (**3b**)*

Reddish brown solid; m.p: 85–87 °C; Anal. calc. for $C_{32}H_{26}FeN_4O_2$: C 69.32, H 4.72; N 10.10%. Found: 69.35, H 4.75; N 10.13%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.51 (s, 1H, Py-H), 8.34 (s, 1H, Ar–H), 8.26 (d, 1H, $J = 7.8$ Hz, Ar–H), 7.92 (d, 1H, $J = 6.9$ Hz, Ar–H), 7.83 (d, 1H, Ar–H), 7.64–7.58 (t, 2H, $J = 7.5$ Hz, Ar–H), 7.4 (s, 1H, Ar–H), 7.2–7.13 (m, 5H), 6.97–6.95 (d, 1H, pyrimidine-H), 4.94 (s, 2H, ferrocene), 4.65 (s, 2H, ferrocene), 4.24 (s, 5H, ferrocene), 2.2 (s, 3H, methyl-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.1, 161.2145.8, 137.8, 137.5, 135.5, 134.2, 130.8, 129.9, 129.3, 127.7, 125.9, 125.6, 124.16, 121.9, 121.6, 118.1 (Ar–C), 84.2, 80.3, 73.1, 70.1, 69.8 (ferrocene-C), 19.7 (methyl-C); IR (KBr, thin film): $\upsilon = 3054$ (Ar–H), 2959 (C–H), 1587 (N=O), 1487 (C=C), 1243 (C–N); MS (EI): $m/z = 554$.

Table 3
The maximum wavelength of absorption spectra of ferrocene tagged multi-functionalized 1,4-DHPMs in methanol.

Compound	λ_{max} for π–π^* transition (nm)	λ_{max} for MLCT (nm)
3a	290	506
3b	270	515
3c	280	515
3d	276	510
3e	288	516
3f	308	506
3g	310	512
3h	310	514
3i	312	510
3j	318	506



Scheme 3. Proposed mechanism of Na/IPA catalyzed cyclocondensation of ferrocenyl chalcones with benzamidines.

*4.2.3. 4-(3-Nitro-phenyl)-6-ferrocenyl-1-pyridin-2-yl-2-(2-chlorophenyl)-1,4-dihydropyrimidine (**3c**)*

Reddish brown solid; m.p: 130–132 °C; Anal. calc. for $C_{31}H_{23}FeN_4O_2Cl$: C 64.77, H 4.02; N 9.74%. Found: C 64.83, H 4.09; N 9.84%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.33 (s, 1H, Py–H), 8.26–8.23 (m, 1H, Ar–H), 7.96–7.92 (m, 1H, Ar–H), 7.89–7.83 (d, 2H, Ar–H), 7.78–7.70 (m, 2H, Ar–H), 7.68–7.58 (m, 2H, Ar–H), 7.41–7.38 (m, 3H, Ar–H), 7.26–7.23 (m, 1H, Ar–H), 6.76–6.74 (d, 1H, pyrimidine-H), 4.95 (s, 2H, ferrocene), 4.66 (s, 2H, ferrocene), 4.23 (d, 5H, ferrocene); ^{13}C NMR (75 MHz, $CDCl_3$): δ 161.1, 158.0, 137.8, 134.2, 130.9, 129.8, 129.4, 129.2, 127.6, 125.3, 123.4, 121.9, 118.6 (Ar–C), 80.5, 72.9, 70.1, 69.7 (ferrocene-C); IR (KBr, thin film): $\upsilon = 3078$ (Ar–

Table 4
In vitro cytotoxicities of ferrocene tagged multi-functionalized 1,4-DHPMs against human breast cancer cell line MDA-MB-435.[a]

Compound	LC_{50}[b]	TGI[c]	GI_{50}[d]
3a	>157.1	138.8	63.2
3b	>144.4	85.3	<18.0
3c	116.5	61.6	<17.4
3d	>147.3	143.0	52.3
3e	>147.3	99.2	40.6
3f	>129.6	125.4	16.85
3g	>129.6	98.5	30.4
3h	>125.5	>125.5	>125.5
3i	>125.5	>125.5	>125.5
3j	>152.9	>152.9	>152.9
Doxorubicin	73.2	<18.4	<18.4

[a] Concentrations in μM.
[b] Concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) calculated from $[(T_i - T_z)/T_z] \times 100 = -50$.
[c] Drug concentration resulting in total growth inhibition (TGI) will calculated from $T_i = T_z$.
[d] Growth inhibition of 50% (GI_{50}) calculated from $[(T_i - T_z)/(C - T_z)] \times 100 = 50$.

H), 2953 (C–H), 1597 (N=O), 1456 (C=C), 1248 (C–N); MS (EI): $m/z = 574$.

4.2.4. 4-(4-Chloro-phenyl)-6-ferrocenyl-2-pyridin-2-yl-1-o-tolyl-1,4-dihydro-pyrimidine (**3d**)

Reddish brown solid; m.p: 124–126 °C; Anal. calc. for $C_{32}H_{26}FeN_3Cl$: C 70.66, H 4.81; N 7.74%. Found: C 70.71, H 4.87; N 7.84%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.60–8.59 (d, 2H, Py-H), 7.88–7.83 (t, 1H, Ar–H), 7.77–7.66 (d, 1H, $J = 6.8$ Hz, Ar–H), 7.59–7.57 (m, 2H, Ar–H), 7.41–7.39 (m, 3H, Ar–H), 7.26–7.24 (m, 2H, Ar–H), 7.11 (s, 1H, Ar–H), 7.06–7.04 (d, 1H, Ar–H), 6.96–6.94 (d, 1H, $J = 6.4$ Hz, pyrimidine-H), 4.91 (s, 2H, ferrocene), 4.61 (s, 2H, ferrocene), 4.22 (s, 5H, ferrocene), 2.2 (s, 3H, methyl-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 161.5, 145.9, 139.3, 137.6, 135.9, 135.6, 133.7, 130.8, 129.4, 129.3, 129.1, 127.7, 125.8, 123.4, 121.4, 118.2 (Ar–C), 80.5, 72.7, 70.0, 69.7 (ferrocene-C), 19.6 (methyl-C); IR (KBr, thin film): υ = 3089 (Ar–H), 2934 (C–H), 1566 (C=N), 1455 (C=C), 1292 (C–N); MS (EI): $m/z = 543$.

4.2.5. 4-(4-Chloro-phenyl)-6-ferrocenyl-1-pyridin-2-yl-2-o-tolyl-1,4-dihydro-pyrimidine (**3e**)

Reddish brown solid; m.p: 134–136 °C; Anal. calc. for $C_{32}H_{26}FeN_3Cl$: C 70.66, H 4.81; N 7.74%. Found: C 70.69, H 4.81; N 7.80%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.38 (s, 1H, Py-H), 7.75–7.70 (m, 4H, Ar–H), 7.58–7.56 (m, 2H, Ar–H), 7.41 (s, 2H, Ar–H), 7.28–7.24 (m, 2H, Ar–H), 7.10–7.05 (m, 2H, Ar–H), 6.97 (d, 1H, $J = 6.4$ Hz, pyrimidine-H), 4.90 (s, 2H, ferrocene), 4.59 (s, 2H, ferrocene), 4.21 (s, 5H, ferrocene), 2.5 (s, 3H, methyl-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 147.7, 139.4, 136.8, 135.8, 133.7, 130.9, 129.4, 129.4, 129.1, 126.9, 125.1, 123.4, 121.0, 114. (Ar–C), 77.4, 77.0, 76.6, 70.0, 69.7 (ferrocene-C), 19.7 (methyl-C); IR (KBr, thin film): υ = 3076 (Ar–H), 2945 (C–H), 1555 (C=N), 1436 (C=C), 1262 (C–N); MS (EI): $m/z = 543$.

4.2.6. 4,6-Diferrocenyl-1-pyridin-2-yl-2-o-tolyl-1,4-dihydro-pyrimidine (**3f**)

Reddish brown solid; m.p: 146 °C; Anal. calc. for $C_{36}H_{31}Fe_2N_3$: C 70.03, H 5.06; N 6.80%. Found: C 70.10, H 5.00; N 6.89%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.3 (d, 1H, $J = 3.4$ Hz, py-H), 7.72–7.70 (d, 2H, $J = 6.5$ Hz, Ar–H), 7.47 (s, 1H, Ar–H), 7.30–7.25 (m, 4H, Ar–H), 6.98 (t, 1H, Ar–H), 6.77–6.75 (d, 1H, $J = 6.4$ Hz, pyrimidine-H), 4.87 (s, 2H, ferrocene), 4.59–4.55 (d, 4H, ferrocene), 4.46 (s, 2H, ferrocene), 4.2–4.18 (d, 10H, ferrocene), 2.5 (s, 3H, methyl-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 145.9, 142.1, 138.2, 135.9, 134.7, 130.9, 130.2, 128.0, 125.9, 120.2, 120.0, 119.3 (Ar–C), 80.8, 79.5, 72.5, 71.0, 70.1,69.7, 69.6, 68.7 (ferrocene-C), 19.8 (methyl-C); IR (KBr, thin film): υ = 3056 (Ar–H), 2976 (C–H), 1535 (C=N), 1425 (C=C), 1237 (C–N); MS (EI): $m/z = 617$.

4.2.7. 4,6-Diferrocenyl-2-pyridin-2-yl-1-o-tolyl-1,4-dihydro-pyrimidine (**3g**)

Reddish brown solid; m.p: 146 °C; Anal. calc. for $C_{36}H_{31}Fe_2N_3$: C 70.03, H 5.06; N 6.80%. Found: C 70.08, H 5.09; N 6.77%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.6 (d, 1H, $J = 4.2$ Hz, py-H), 8.5 (d, 1H, $J = 7.2$ Hz, Ar–H), 7.8 (t, 1H, Ar–H), 7.7 (d, 1H, Ar–H), 7.43–7.40 (t, 1H, Ar–H), 7.26–7.21 (m, 2H, Ar–H), 7.0 (t, 1H, Ar–H), 6.9 (d, 1H, Ar–H), 6.77–6.75 (d, 1H, $J = 6.4$ Hz, pyrimidine-H), 4.8 (s, 2H, ferrocene), 4.6 (d, 2H, ferrocene), 4.5 (s, 2H, ferrocene), 4.47 (s, 2H, ferrocene), 4.2–4.18 (d, 10H, ferrocene), 2.2 (s, 3H, methyl-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 147.9, 142.0, 136.9, 130.8, 126.9, 125.3, 121.9, 120.3, (Ar–C), 80.8, 79.5, 72.4, 70.9, 70.0,69.7, 69.6, 68.7 (ferrocene-C), 17.6 (methyl-C); IR (KBr, thin film): υ = 3074 (Ar–H), 2985 (C–H), 1542 (C=N), 1446 (C=C), 1259 (C–N); MS (EI): $m/z = 617$.

4.2.8. 2-(2-Chloro-phenyl)-4,6-diferrocenyl-1-pyridin-2-yl-1,4-dihydro-pyrimidine (**3h**)

Dark brown solid; m.p: 160–162 °C; Anal. calc. for $C_{35}H_{28}Fe_2N_3Cl$: C 65.91, H 4.42; N 6.58%. Found: C 65.89, H 4.38; N 6.53%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.36 (s, 1H, py-H), 7.98 (s, 1H, Ar–H), 7.84 (s, 1H, Ar–H), 7.74–7.72 (d, 2H, $J = 6.6$ Hz, Ar–H), 7.48–7.42 (m, 3H, Ar–H), 7.0 (s, 1H, Ar–H), 6.77–6.75 (d, 1H, $J = 6.3$ Hz, pyrimidine-H), 4.8 (s, 2H, ferrocene), 4.6–4.5 (d, 4H, ferrocene), 4.4 (s, 2H, ferrocene), 4.2–4.18 (d, 10H, ferrocene); ^{13}C NMR (75 MHz, $CDCl_3$): δ 145.9, 142.1, 138, 134.6, 131.3, 129.9, 127.7, 125.6, 121.3, 120.2, 118.9 (Ar–C), 80.7, 79.5, 72.5, 71.0, 70.1,69.7, 69.6, 68.7 (ferrocene-C); IR (KBr, thin film): υ = 3076 (Ar–H), 2965 (C–H), 1537 (C=N), 1432 (C=C), 1268 (C–N); MS (EI): $m/z = 637$.

4.2.9. 2-(3-Chloro-phenyl)-4,6-diferrocenyl-1-pyridin-2-yl-1,4-dihydro-pyrimidine (**3i**)

Reddish brown solid; m.p: 170–172 °C; Anal. calc. for $C_{35}H_{28}Fe_2N_3Cl$: C 65.91, H 4.42; N 6.58%. Found: C 65.93, H 4.46; N 6.62%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.36 (s, 1H, py-H), 7.98 (s, 1H, Ar–H), 7.83 (s, 1H, Ar–H), 7.74–7.71 (d, 2H, $J = 6.6$ Hz, Ar–H), 7.44 (m, 3H,Ar–H), 7.0 (s, 1H, Ar–H), 6.76–6.74 (d, 1H, $J = 6.3$ Hz, pyrimidine-H), 4.8 (s, 2H, ferrocene), 4.6–4.5 (d, 4H, ferrocene), 4.4 (s, 2H, ferrocene), 4.2–4.19 (d, 10H, ferrocene); ^{13}C NMR (75 MHz, $CDCl_3$): δ 145.9, 142.1, 137.9, 134.7, 131.2, 129.9, 127.7, 125.5, 121.5,12.3, 118.85, (Ar–C), 80.7, 79.5, 72.5, 71.0, 70.1, 69.7, 69.6, 68.8 (ferrocene-C); IR (KBr, thin film): υ = 3052 (Ar–H), 2960 (C–H), 1553 (C=N), 1490 (C=C), 1242 (C–N); MS (EI): $m/z = 637$.

4.2.10. 6-Ferrocenyl-1-pyridin-2-yl-2-o-tolyl-4-p-tolyl-1,4-dihydro-pyrimidin (**3j**)

Reddish brown solid; m.p: 154–156 °C; Anal. calc. for $C_{33}H_{29}FeN_3$: C 75.71, H 5.58; N 8.02%. Found: C 75.70, H 5.52; N 8.00%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.53 (d, 2H, $J = 4.2$ Hz, py-H), 8.51–8.49 (d, 1H, $J = 7.5$ Hz, Ar–H), 7.85–7.75 (m, 2H, Ar–H), 7.56 (d, 2H, $J = 7.8$ Hz, Ar–H), 7.42–7.38 (t, 1H, Ar–H), 7.28–7.17 (m, 3H, Ar–H), 7.11–7.09 (d, 1H, $J = 7.8$ Hz, Ar–H), 7.02–6.97 (t, 1H, Ar–H), 6.92–6.90 (d, 1H, $J = 6.6$ Hz, pyrimidine-H), 4.91 (s, 2H, ferrocene), 4.56 (s, 2H, ferrocene), 4.21 (s, 5H, ferrocene), 2.41 (s, 3H, methyl-H), 2.20 (s, 3H, methyl-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 151.4, 147.8, 140.9, 140.3, 136.6, 132.5, 130.8, 129.6, 128.3, 126.8, 125.0, 123.2, 121.9, 121.7, 120.7 (Ar–C), 80.7, 72.5, 70.0,69.7, 69.7(ferrocene-C), 21.5, 17.7 (methyl-C); IR (KBr, thin film): υ = 3057 (Ar–H), 2949 (C–H), 1542 (C=N), 1451 (C=C), 1249 (C–N); MS (EI): $m/z = 523$.

4.3. Procedure of the SRB-assay

Tumor cells (human breast cancer cell line MDA-MB-435) were grown in tissue culture flasks in growth medium (RPMI-1640 with 2 mM glutamine, pH 7.4, 10% fetal calf serum, 100 μg/mL streptomycin, and 100 units/mL penicillin) at 37 °C under the atmosphere of 5% CO_2 and 95% relative humidity employing a CO_2 incubator. The cells at subconfluent stage were harvested from the flask by treatment with trypsin (0.05% trypsin in PBS containing 0.02% EDTA) and placed in growth medium. The cells with more than 97% viability (trypan blue exclusion) were used for cytotoxicity studies. An aliquot of 100 μL of cells were transferred to a well of 96-well tissue culture plate. The cells were allowed to grow for one day at 37 °C in a CO_2 incubator as mentioned above. The test materials at different concentrations were then added to the wells and cells were further allowed to grow for another 48 h. Suitable blanks and positive controls were also included. Each test was performed in triplicate. The cell growth was stopped by gently layering of 50 μL of 50% trichloroacetic acid. The plates were incubated at 4 °C for an hour to fix the cells attached to the bottom of the wells. Liquids of all the wells were gently pipette out and discarded. The plates were washed five times with doubly distilled water to remove TCA, growth medium, etc and were air-dried. 100 μL of SRB solution (0.4% in 1% acetic acid) was added to each well and the plates were incubated at ambient temperature

for half an hour. The unbound SRB was quickly removed by washing the wells five times with 1% acetic acid. Plates were air dried, tris-buffer (100 μL of 0.01 M, pH 10.4) was added to all the wells and plates were gently stirred for 5 min on a mechanical stirrer. The optical density was measured on ELISA reader at 540 nm. The cell growth at absence of any test material was considered 100% and in turn growth inhibition was calculated. GI_{50} values were determined by regression analysis.

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References

[1] T.J. Kealy, P.L. Pauson, Nature 168 (1951) 1039–1040.
[2] (a) A. Togni, T. Hayashi, Ferrocenes: Homogeneous Catalysis, Organic Synthesis, Material Science, VCH, Weinheim, 1995;
(b) R. Atkinson, V. Gibson, N. Long, Chem. Soc. Rev. 33 (2004) 313–328;
(c) D.L. Xin, H.X. Long, Chiral Ferrocenes in Asymmetric Catalysis: Synthesis and Applications, Wiley-VCH Verlag, GmbH & Co. KGaA, Weinheim, 2010;
(d) H. Werner, Angew. Chem. Int. Ed. 51 (2012) 6052–6058 (and references cited therein).
[3] N. Metzler-Nolte, M. Salmain, in: P. Stepnicka (Ed.), Ferrocenes: Ligands, Materials and Biomolecules, Wiley, New York, 2008, pp. 499–639.
[4] (a) C. Ornelas, J.R. Aranzaes, E. Cloutet, S. Alves, D. Astruc, Angew. Chem. Int. Ed. 46 (2007) 872–877;
(b) R. Djeda, A. Rapakousiou, L. Liang, N. Guidolin, J. Ruiz, D. Astruc, Angew. Chem. Int. Ed. 49 (2010) 8152–8156.
[5] I.R. Whittall, A.M. McDonagh, M.G. Humphrey, M. Samoc, Adv. Organomet. Chem. 42 (1998) 291–362.
[6] (a) D.R. van Staveren, N. Metzler-Nolte, Chem. Rev. 104 (2004) 5931–5985;
(b) M.F.R. Fouda, M.M. Abd-Elzaher, R.A. Abdelsamaia, A.A. Labib, Appl. Organomet. Chem. 21 (2007) 613–625.
[7] (a) V. Ganesh, V.S. Sudhir, T. Kundu, S. Chandrasekaran, Chem. Asian J. 6 (2011) 2670–2694;
(b) N.H. Evans, P.D. Beer, Org. Biomol. Chem. 9 (2011) 92–100;
(c) R. Gómez Arrayás, J. Adrio, J.C. Carretero, Angew. Chem. Int. Ed. 45 (2006) 7674–7715;
(d) H.B. Kraatz, J. Inorg, Organomet. Polym. Mater. 15 (2005) 83–106;
(e) J.C. Thomas, Chem. Rev. 103 (2003) 310;
(f) L.-X. Dai, T. Tu, S.-L. You, W.-P. Deng, X.-L. Hou, Acc. Chem. Res. 36 (2003) 659–667;
(g) C.J. Richards, A.J. Locke, Tetrahedron Asymmetry 9 (1998) 2377–2407;
(h) A. Togni, Angew. Chem. Int. Ed. 35 (1996) 1475–1477.
[8] D. Astruc, Electron Transfer and Radical Processes in Transition-metal Chemistry, VCH, New York, 1995.
[9] A. Nguyen, A. Vessiéres, E.A. Hillard, S. Top, P. Pigeon, G. Jaouen, Chimia 61 (2007) 716–724.
[10] (a) C. Ornelas, New J. Chem. 35 (2011) 1973–1985;
(b) D. Plazuk, B. Rychlik, A. Błauz, S. Domagała, J. Organomet. Chem. 715 (2012) 102–112;
(c) A. Hottin, F. Dubar, A. Steenackers, P. Delannoy, C. Biot, J.B. Behr, Org. Biomol. Chem. 10 (2012) 5592–5597;
(d) G. Gasser, I. Ott, N. Metzler-Nolte, J. Med. Chem. 54 (2011) 3–25;
(e) K. Kowalski, A. Koceva-Chyla, A. Pieniążek, J. Bernasińska, J. Skiba, A.J. Rybarczyk-Pirek, Z. Jóźwiak, J. Organomet. Chem. 700 (2012) 58–68;
(f) K.N. Tiwari, J.P. Monserrat, A. Hequet, C. Ganem-Elbaz, T. Cresteil, G. Jaouen, A. Vessières, E.A. Hillard, C. Jolivalt, Dalton Trans. 41 (2012) 6451–6457;
(g) A.C. De Oliveira, E.A. Hillard, P. Pigeon, D.D. Rocha, F.A.R. Rodrigues, R.C. Montenegro, L.V. Costa-Lotufo, M.O.F. Goulart, G. Jaouen, Eur. J. Med. Chem. 46 (2011) 3778–3787;
(h) B. Long, S. Liang, D. Xin, Y. Yang, J. Xiang, Eur. J. Med. Chem. 44 (2009) 2572–2576;
(i) J.C. Swarts, D.M. Swarts, D.M. Maree, E.W. Neuse, C. La Madeleine, J.E. Van Lier, Anticancer Res. 21 (2001) 2033–2037.
[11] (a) C.O. Kappe, Tetrahedron 49 (1993) 6937–6963;
(b) C.O. Kappe, Eur. J. Med. Chem. 35 (2000) 1043–1052.
[12] H. Cho, M. Ueda, K. Shima, A. Mizuno, M. Hayashimatsu, Y. Ohnaka, Y. Takeuchi, M. Hamaguchi, K. Aisaka, J. Med. Chem. 32 (1989) 2399–2406.
[13] D. Nagarathnam, S.W. Miao, B. Lagu, G. Chiu, J. Fang, T.G.M. Dhar, J. Zhang, S. Tyagarajan, M.R. Marzabadi, F.Q. Zhang, W.C. Wong, W.Y. Sun, D. Tian, J.M. Wetzel, C. Forray, R.S.L. Chnag, T.P. Broten, R.W. Ransom, T.W. Schorn, T.B. Chen, S. O'Malley, P. Kling, K. Schneck, R. Benedesky, C.M. Harrell, K.P. Vyas, C. Gluchowski, J. Med. Chem. 42 (1999) 4764–4777.
[14] A.D. Patil, N.V. Kumar, W.C. Kokke, M.F. Bean, A.J. Freyer, C. De Brosse, S. Mai, A. Truneh, D.J. Faulkner, B. Carte, A.L. Breen, R.P. Hertzberg, R.K. Johnson, J.W. Westley, B.C.M. Ports, J. Org. Chem. 60 (1995) 1182–1188.
[15] K. Deres, C.H. Schröder, A. Paessens, S. Goldmann, H.J. Hacker, O. Weber, T. Krämer, U. Niewöhner, U. Pleiss, J. Stoltefuss, E. Graef, D. Koletzki, R.N.A. Masantschek, A. Reimann, R. Jaeger, R. Gross, B. Beckermann, K.H. Schlemmer, D. Haebich, H. Rübsamen-Waigmann, Science 299 (2003) 893–896.
[16] (a) C.A. Sehon, G.Z. Wang, A.Q. Viet, K.B. Goodman, S.E. Dowdell, P.A. Elkins, S.F. Semus, C. Evans, L.J. Jolivette, R.B. Kirkpatrick, E. Dul, S.S. Khandekar, T. Yi, L.L. Wright, G.K. Smith, D.J. Behm, R. Bentley, C.P. Doe, E. Hu, D. Lee, J. Med. Chem. 51 (2008) 6631–6634;
(b) Q. Sun, F. Suzenet, G. Guillaumet, Tetrahedron Lett. 53 (2012) 2694–2698;
(c) V.B. Jadhav, H.V. Holla, S.U. Tekale, R.P. Pawar, Der Chem. Sin. 3 (2012) 1213–1228.
[17] (a) T.U. Mayer, T.M. Kapoor, S.J. Haggarty, R.W. King, S.L. Schreiber, T.J. Mitchison, Science 286 (1999) 971–974;
(b) S.J. Haggarty, T.U. Mayer, D.T. Miyamoto, R. Fathi, R.W. King, T.J. Mitchison, S.L. Schreiber, Chem. Biol. 7 (2000) 275–286.
[18] (a) G. Rashinkar, R. Salunkhe, J. Mol. Catal. A: Chem. 316 (2010) 146–152;
(b) G. Rashinkar, S. Kamble, A. Kumbhar, R. Salunkhe, Catal. Commun. 12 (2011) 1442–1447;
(c) R. Kurane, V. Gaikwad, J. Jadhav, R. Salunkhe, G. Rashinkar, Tetrahedron Lett. 53 (2012) 6361–6366.
[19] X. Wu, P. Wilairat, M.L. Go, Bioorg. Med. Chem. Lett. 12 (2002) 2299–2302.
[20] (a) T.J. Muller, J. Conradie, E. Erasmus, Polyhedron 33 (2012) 257–266;
(b) H. Parveen, F. Hayat, A. Salahuddin, A. Azam, Eur. J. Med. Chem. 45 (2010) 3497–3503;
(c) S. Arya, N. Kumar, P. Roy, S.M. Sondhi, Eur. J. Med. Chem. 59 (2013) 7–14.
[21] (a) J. Zienkiewicz, V.G. Young, P. Kaszynski, J. Org. Chem. 69 (2004) 2551;
(b) A.A. Aly, A.M. Nour-El-Din, ARKIVOC i (2008) 153–194.
[22] P. Skehn, R. Storeng, A. Scudiero, J. Monks, D. McMohan, D. Vistica, T.W. Jonathan, H. Bokesch, S. Kenney, M.R. Boyd, J. Natl. Cancer Inst. 82 (1990) 1107–1112.