Pergamon

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Bioorganic & Medicinal Chemistry Letters 13 (2003) 2017–2020

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

N-Ferrocenylmethyl, *N*′-Methyl-2-substituted Benzimidazolium Iodide Salts with In Vitro Activity Against the *Leishmania infantum* Parasite Strain L1

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Received 2 December 2002; revised 10 February 2003; accepted 19 March 2003

Abstract—Herein, we disclose results of our research into a class of benzimidazolium compounds active against the *Leishmania infantum* parasite strain L1. We have discovered that *N*-ferrocenylmethyl, *N*′-methyl-2-aryl (or styryl) benzimidazolium iodide salts show in vitro activity against this strain.

The World Health Organisation (WHO) lists three diseases caused by kinetoplastids as being of primary importance in tropical and subtropical regions of the world; African trypanosomiasis, the leishmaniases and Chagas disease.[1] Chemotherapy for clinical disease and prophylaxis in animals remains a primary concern. Since most available drugs have been in use for more than 40 years and are toxic while resistance to most of the primary chemotherapeutic agents has increased, it is imperative that new classes of compounds, potential drugs, are designed, synthesised, and tested.

Leishmaniasis is an old but largely unknown disease. Today, this disease affects 12 million people in 88 countries. The majority of cases are in developing countries, especially in the poorest and most remote communities. Unacknowledged and uncontrolled, this treatable disease continues to claim lives in remote areas. The parasite is transmitted by a sand fly living in tropical and temperate regions. The disease affects animals such as rodents and dogs, as well as humans.

In humans the disease exists in different forms depending on the type of parasite and the immunity of the infected person. Thus it may present as simple skin ulcers, or in a progressive form that may permanently disfigure those infected, or as visceral leishmaniasis which invades the whole body.

Classical treatment of leishmaniasis involves the antimony based drug, amphohotericine B (ampho-B). However, with increasing resistance of the parasite to this drug new treatments are required. There are few leading experimental drugs, ketaconazole **1** an inhibitor of cytochrome P450[2] and miltefone (or hexadecylphosphocholine) **2**[3] are two of the more promising. Ketoconazole is based on an azole group and through our investigations into novel azoles as anion receptors we had produced a series of benzimidazoles and corresponding iodide salts.[4] This, coupled to our modest success in the use of *N*-ferrocenylmethyl, *N*′-methyl-2-substituted benzimidazolium iodide salts against the malaria parasite strain NF54 in vitro,[5] warranted testing these same compounds against the *Leishmania infantum* parasite strain L1 in vitro. It should be noted that these molecules also bear similarity to recent work by Brocard based on the fact that the parasite needs iron for its development inside the red blood cell. Brocard and coworkers decided to try a simple strategy: combine poison (chloroquine) and bait (ferrocene) in the same molecule. They inserted a ferrocenyl group into the side chain of chloroquine, thus producing a hybrid compound called ferroquine that is much more potent in mice than chloroquine.[6]

The compounds **5a–i**, **6a–i**, **8a–e** and **9a–e** were synthesised according to Schemes 1 and 2, and the general

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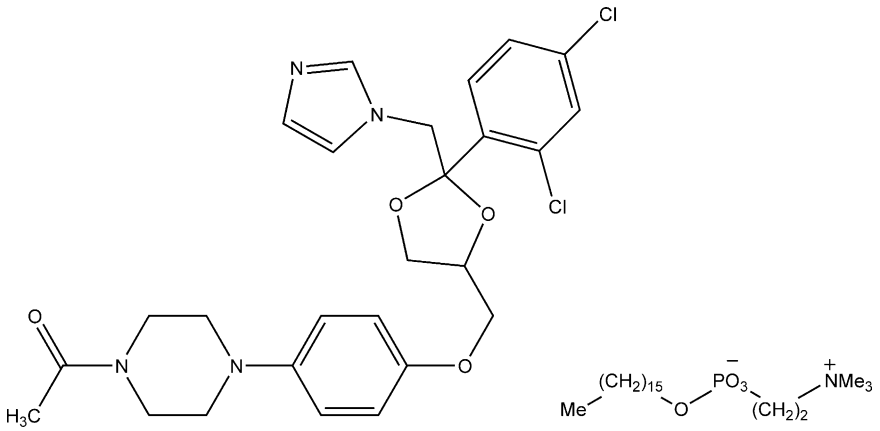
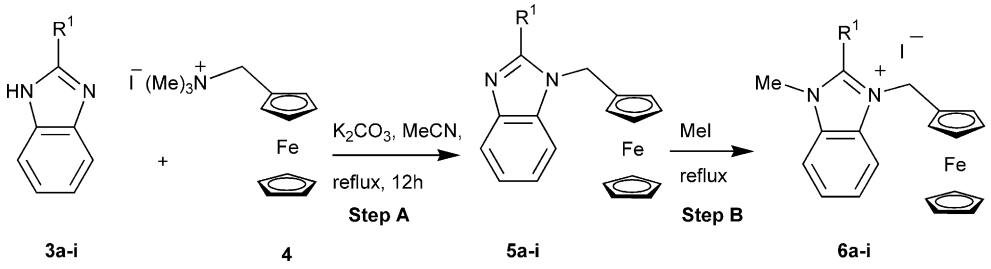
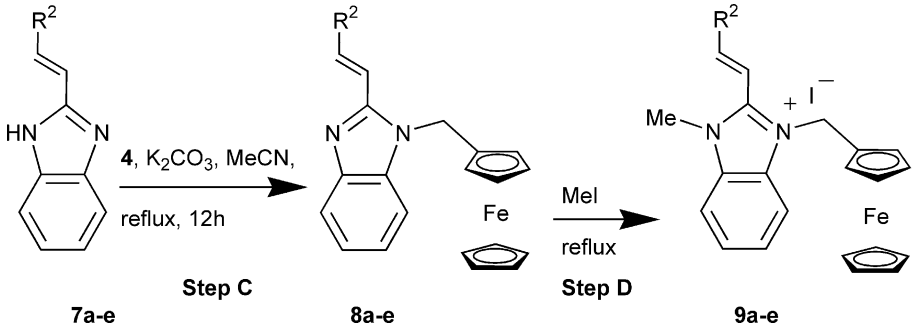
0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00327-5



1 2



Scheme 1.

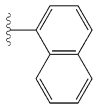
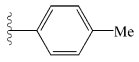
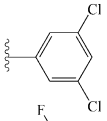
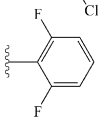
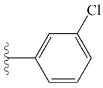
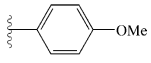


Scheme 2.

synthetic methods are given in refs 7 and 8. The results are given in Tables 1 and 2.

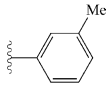
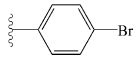
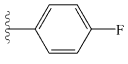
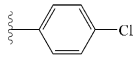
From Tables 1 and 2, it can immediately be seen that 10 out of the 28 compounds tested by the WHO show activity against the *L. infantum* parasite strain L1, with IC_{50}'s in the range <0.5 to >6.25 μM. Although the most active compounds **9a** and **9c** are at least 25 times less active than the current drug of choice PX-6518 these compounds based on *N*-ferrocenylmethyl, *N*′-methyl-2-substituted benzimidazoles and benzimidazolium iodide salts are clearly interesting lead compounds with possibilities for development as drugs active against the *L. infantum* parasite strain L1. We are currently investigating these possibilities and likely modes of action of such compounds. This family of compounds also appears to have antibacterial and anticancer activity which we are currently assessing.

Table 1. Benzimidazoles **5** and salts **6** against the *Leishmania infantum* parasite strain L1

Compd	R^1	Yield (%)		IC_{50} μM, benzimidazole 5 L1	IC_{50} μM, salt 6 L1
		Step A	Step B		
3/5/6a		67	94	—	>12.50
b		73	99	>12.50	1.56
c		63	96	>12.50	6.50
d		71	91	>12.50	>12.50
e		73	93	>12.50	6.00
f		76	92	>12.50	11.00
g		75	80	>12.50	2.20
h		64	84	>12.50	4.00
i		54	87	—	1.56

Standards: Ampho-B < 6.25 μM, PX-6518 < 0.02 μM.

Table 2. Benzimidazoles **8** and salts **9** against the *Leishmania infantum* parasite strain L1

Compd	R^2	Yield (%)		IC_{50} mM, benzimidazole 8 L1	IC_{50} mM, salt 9 L1
		Step C	Step D		
7/8/9 a	Me	67	90	25.00	14.00
b		73	85	32.00	0.50
c		63	82	32.00	0.50
d		71	64	6.00	2.00
e		73	73	15.00	1.00

Standards: Ampho-B < 6.25 μM, PX-6518 < 0.02 μM.

Acknowledgements

The authors would like to acknowledge the contribution to this research by the Tropical Disease Research section of the World Health Organisation, Enterprise Ireland, and Arch Chemicals BV.

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7. General experimental for aryl series **6a–i** using the synthesis of **6a** as an example. To a mixture of 2-phenylbenzimidazole[9] **3a** (2.5 g, 13.0 mmol) and potassium carbonate (2.7 g, 19.5 mmol) in acetonitrile (150 mL) was added (trimethylammonium)ferrocenylmethyl iodide **4** (10.0 g, 13.0 mmol). The mixture was heated at reflux for 12 h, cooled to room temperature and water was added. The resulting suspension was extracted into chloroform, the organic layer was washed with water, dried over magnesium sulfate and evaporated under vacuum to leave an orange gum. The crude product was purified by column chromatography on silica gel using DCM:methanol (97:3) as eluent. Compound **5a** was obtained as a light orange solid. *N*-ferrocenylmethyl-2-(phenyl)benzimidazole **5a** (1.0 g, 2.6 mmol) was stirred at room temperature with an excess of methyl iodide (5 mL). After approx. 20 min a fine yellow ppt. fell out of solution. The mixture was heated for 2 h, filtered and the precipitate washed with ether. Compound **6a** was obtained as a light yellow powder. All the aryl series **5a–i** and **6a–i**, or hexafluorophosphate salts thereof, gave correct analytical data. Data for compounds **5a–i**: **5a**, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.83 (m, 2H, aryl-H), 7.73 (m, 1H, aryl-H), 7.65 (m, 4H, aryl-H), 7.25 (m, 2H, aryl-H), 5.20 (s, 2H, Fc-CH_2), 4.17 (m, 2H, cpd-H), 4.07 (s, 5H, cpd-H), 4.04 (m, 2H, cpd-H); ^{13}C (100MHz, $CDCl_3$) δ (ppm) 153.2, 138.1, 135.5, 131.3, 130.6, 127.1, 122.4, 122.05, 119.1, 111.3, 82.9, 69.0, 68.8, 68.2, 43.6; IR (KBr) ν (cm^{-1}) 3016, 1724, 1658, 1459, 1329, 1215, 1105, 825, 755; m.p 132–136 °C. **5b**, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.19 (s, 1H, aryl-H), 7.93 (m, 1H, aryl-H), 7.86 (m, 2H, aryl-H), 7.78 (m, 2H, aryl-H), 7.51 (m, 2H, aryl-H), 7.47 (m, 1H, aryl-H), 7.24 (m, 2H, aryl-H), 5.18 (s, 2H, Fc-CH_2), 4.00 (m, 9H, cpd-H); ^{13}C (100 MHz, $CDCl_3$) δ (ppm) 153.9, 142.5, 136.2, 134.1, 133.4, 130.0, 128.9, 128.8, 128.3, 127.6, 127.2, 126.9, 123.2, 122.9, 120.4, 110.8, 69.2, 68.6, 45.1; IR (KBr) ν (cm^{-1}) 3040, 2960, 2285, 1708, 1633, 1502, 1452, 1410, 1387, 1325, 1262, 1153, 1107, 1040, 1029, 950, 895, 825, 746, 695; m.p 176–180 °C. **5c**, ^{1}H NMR (400MHz, $CDCl_3$) δ (ppm) 7.80 (m, 1H, aryl-H), 7.64 (m, 1H, thienyl-H), 7.56 (m, 1H, thienyl-H), 7.47 (m, 1H, aryl-H), 7.32 (m, 2H, aryl-H), 7.23 (m, 1H, thienyl-H), 5.35 (s, 2H, Fc-CH_2), 4.19 (m, 2H, cpd-H), 4.15 (m, 5H, cpd-H), 4.11 (m, 2H, cpd-H); ^{13}C (100 MHz, $CDCl_3$) δ (ppm) 147.6, 143.3, 136.3, 132.8, 128.6, 123.9, 123.3, 123.1, 120.2, 110.6, 83.7, 69.8, 69.3, 69.0, 68.7, 44.8; IR (KBr) ν (cm^{-1}) 3040, 2971, 2297, 1702, 1639, 1605, 1554, 1444, 1416, 1362, 1325, 1210, 1147, 1107, 1040, 1004, 901, 844, 731, 689; m.p 130–134 °C. **5d**, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.82 (m, 1H, aryl-H), 7.69 (m, 2H, aryl-H), 7.46 (m, 1H, aryl-H), 7.39 (m, 2H, aryl-H), 7.29 (m, 2H, aryl-H), 5.22 (s, 2H, Fc-CH_2), 4.18–4.07 (m, 9H, cpd-H), 2.49 (s, 3H, aryl-CH_3); ^{13}C (100MHz, $CDCl_3$) δ (ppm) 153.6, 142.9, 139.8, 135.6, 129.5, 127.7, 122.5, 122.2, 119.8, 110.3, 83.2, 68.7, 68.5, 68.1, 44.3, 21.5; IR (KBr) ν (cm^{-1}) 3051, 2971, 2685, 2297, 1713, 1616, 1479, 1450, 1416, 1381, 1324, 1261, 1158, 1107, 1015, 1005, 895, 821, 741, 706; m.p 158–162 °C. **5e**,

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.73 (m, 1H, aryl-H), 7.63 (m, 2H, aryl-H), 7.45 (m, 1H, aryl-H), 7.42 (m, 1H, aryl-H), 7.27 (m, 2H, aryl-H), 5.10 (s, 2H, Fc-CH_2), 4.07–4.05 (m, 9H, cpd-H; ^{13}C (100 MHz, $CDCl_3$) δ (ppm) 150.6, 142.9, 135.9, 135.6, 133.6, 129.9, 128.1, 123.6, 123.0, 120.4, 110.7, 82.9, 69.1, 68.9, 68.6, 44.8; IR (KBr) ν (cm^{-1}) 3062, 2982, 2685, 2285, 1708, 1605, 1559, 1450, 1444, 1357, 1346, 1330, 1256, 1164, 1107, 1027, 992, 889, 856, 798, 741, 706; m.p 148–152 °C. **5f**, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.87 (m, 1H, aryl-H), 7.57 (m, 1H, aryl-H), 7.49 (m, 1H, aryl-H), 7.33 (m, 2H, aryl-H), 7.14 (m, 2H, aryl-H), 5.06 (s, 2H, Fc-CH_2), 4.04–3.98 (m, 9H, cpd-H); ^{13}C (100 MHz, $CDCl_3$) δ (ppm) 162.4, 162.3, 159.9, 159.7, 143.3, 142.2, 134.7, 132.5, 132.4, 132.3, 123.1, 122.3, 120.3, 112.0, 111.9, 111.8, 111.7, 110.3, 109.2, 109.0, 108.8, 82.1, 68.7, 68.6, 68.3, 44.2; IR (KBr) ν (cm^{-1}) 3040, 2971, 2297, 1708, 1639, 1473, 1444, 1416, 1387, 1330, 1261, 1153, 1107, 1047, 1039, 1004, 895, 817, 795, 741, 700; M.p 204–206 °C. **5g**, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.68 (m, 2H, aryl-H), 7.39 (m, 4H, aryl-H), 7.22 (m, 2H, aryl-H), 4.92 (s, 2H, Fc-CH_2), 3.97–3.85 (m, 9H, cpd-H); ^{13}C (100 MHz, $CDCl_3$) δ (ppm) 152.0, 143.2, 134.9, 133.3, 133.2, 132.9, 131.9, 127.79, 124.2, 123.3, 122.7, 120.6, 110.7, 82.7, 69.3, 69.1, 68.7, 44.6; IR (KBr) ν (cm^{-1}) 3051, 2982, 2308, 1708, 1639, 1563, 1525, 1450, 1393, 1324, 1261, 1153, 1101, 1027, 1004, 941, 895, 826, 735, 700, 667; M.p 138–142 °C. **5h**, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.82 (m, 2H, aryl-H), 7.69 (m, 1H, aryl-H), 7.56–7.48 (m, 3H, aryl-H), 7.34 (m, 2H, aryl-H), 5.22 (s, 2H, Fc-CH_2), 4.13–4.07 (m, 9H, cpd-H); ^{13}C (100 MHz, $CDCl_3$) δ (ppm) 152.3, 143.3, 136.0, 135.1, 132.8, 130.4, 130.3, 130.1, 128.2, 123.5, 123.1, 120.5, 110.9, 83.4, 69.3, 69.1, 68.7, 44.9; IR (KBr) ν (cm^{-1}) 3051, 2971, 2308, 1708, 1639, 1456, 1422, 1364, 1330, 1261, 1153, 1101, 1027, 1008, 895, 741, 706, 695; M.p 160–164 °C. **5i**, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.81 (m, 1H, aryl-H), 7.71 (m, 2H, aryl-H), 7.45 (m, 1H, aryl-H), 7.30 (m, 2H, aryl-H), 7.09 (m, 2H, aryl-H), 5.21 (s, 2H, Fc-CH_2), 4.21–4.07 (m, 9H, cpd-H), 3.91 (s, 3H, aryl-OCH_3); ^{13}C (100 MHz, $CDCl_3$) δ (ppm) 160.8, 153.5, 142.9, 135.6, 131.0, 122.9, 122.4, 119.6, 114.1, 110.3, 83.3, 68.8, 68.1, 55.4, 44.3; IR (KBr) ν (cm^{-1}) 3043, 2981, 2844, 2310, 1708, 1609, 1495, 1434, 1420, 1363, 1259, 1145, 1024, 898, 743, 709; M.p 142–146 °C.

8. General experimental for styryl series **9a–e** using the synthesis of **9a** as an example. To a mixture of 2-propenylbenzimidazole[10] **7a** (3.0 g, 19.0 mmol) and potassium carbonate (3.4 g, 28.5 mmol) in acetonitrile (100mL) was added (trimethylammonium)ferrocenylmethyl iodide **4** (7.3 g, 19.0 mmol). The mixture was heated at reflux for 12 h, cooled to room temperature and water added. The resulting suspension was extracted into chloroform, the organic layer was washed with water, dried over magnesium sulfate and evaporated under vacuum to leave a dark brown solid. The crude product was purified by column chromatography on silica gel using DCM:methanol (97:3) as eluent. The compound **8a** was obtained as a light orange solid. *N*-Ferrocenylmethyl-2-(3-methylstyryl)benzimidazole **8a** (1.3 g, 3.0 mmol) was refluxed in excess methyl iodide (7 mL) for 2 h. The resultant orange precipitate was filtered and washed with ether. Compound **9a** was obtained as an orange powder. All styryl series **9a–e**, or hexafluorophosphate salts thereof, gave correct analytical data. Data for compounds **8a–e**: **8a**, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.72 (m, 1H, aryl-H), 7.37 (m, 1H, aryl-H), 7.25 (m, 2H, aryl-H), 7.19 (m, 1H, vinylic-H), 6.59 (d, 1H, vinylic-H, J = 15.6 Hz), 5.05 (s, 2H, Fc-CH_2), 4.23 (m, 2H, cpd-H), 4.17 (s, 5H, cpd-H), 4.11 (m, 2H, cpd-H), 2.06 (d, 3H, CH_3, J = 6.8 Hz); ^{13}C (100 MHz, $CDCl_3$) δ (ppm) 150.9, 143.3, 136.9, 135.3, 122.7, 122.5, 119.6, 117.7, 109.9, 83.8, 68.9, 68.7, 43.2, 19.5; IR (KBr) ν (cm^{-1}) 3045, 2969, 1665, 1509, 1477, 1472, 1113, 969, 743, 702; M.p 116–120 °C. **8b**, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.90 (d, 1H, C*H*=CH, J = 15.6 Hz), 7.69 (m, 1H, aryl-H), 7.33 (m, 3H, aryl-H), 7.19 (m, 3H, aryl-H), 7.10 (m, 2H, CH=CH overlapping aryl-H), 5.06 (s, 2H, Fc-CH_2), 4.16 (m, 2H, cpd-H), 4.07 (m, 7H, cpd-H), 2.32 (s, 3H, CH_3); ^{13}C (100 MHz, $CDCl_3$) δ (ppm) 150.9, 143.5, 138.9, 137.7, 136.5, 135.6, 130.4, 129.2, 128.4, 124.7, 123.0, 122.9, 119.8, 113.6, 109.9, 83.9, 69.2, 68.9, 68.8, 43.3, 21.9; IR (KBr) ν (cm^{-1}) 3062, 2982, 2925, 2685, 2308, 1713, 1632, 1604, 1581, 1495, 1454, 1397, 1328, 1265, 1219, 1156, 1104, 1041, 1001, 967, 892, 823, 789, 737, 703; M.p 156–160 °C. **8c**, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.87 (d, 1H, C*H*=CH, J = 16.0 Hz), 7.69 (m, 1H, aryl-H), 7.47 (d, 2H, aryl-H, J = 8.4 Hz), 7.40 (d, 2H, aryl-H, J = 8.4 Hz), 7.37 (m, 1H, aryl-H), 7.21 (m, 2H, aryl-H), 7.11 (d, 1H, CH=*CH*, J = 16 Hz), 5.09 (s, 2H, Fc-CH_2), 4.17 (m, 2H, cpd-H), 4.09 (s, 5H, cpd-H), 4.05 (m, 2H, cpd-H); ^{13}C (100 MHz, $CDCl_3$) δ (ppm) 150.5, 143.5, 136.0, 135.7, 135.4, 132.5, 129.0, 123.4, 123.2, 123.1, 119.9, 118.4, 109.9, 83.9, 69.3, 68.9, 68.8, 43.4; IR (KBr) ν (cm^{-1}) 3062, 2982, 2320, 1713, 1638, 1489, 1449, 1414, 1386, 1322, 1265, 1156, 1104, 1064, 1007, 961, 892, 812, 743, 703, 660; M.p 162–166 °C. **8d**, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.92–7.18 (m, 10H, aryl-H overlapping vinylic-H), 5.41 (s, 2H, Fc-CH_2), 4.32 (m, 2H, cpd-H), 4.26 (s, 5H, cpd-H), 4.07 (m, 2H, cpd-H); ^{13}C (100 MHz, $CDCl_3$) δ (ppm) 164.0, 161.6, 150.5, 143.1, 135.4, 134.9, 133.0, 132.9, 130.0, 129.9, 122.5, 122.4, 118.8, 116.3, 116.0, 114.8, 110.9, 84.5, 69.8, 69.0, 68.3, 43.4; IR (KBr) ν (cm^{-1}) 3051, 2960, 2914, 2114, 1890, 1713, 1644, 1598, 1506, 1454, 1420, 1391, 1334, 1294, 1231, 1156, 1110, 1041, 1009, 972, 915, 897, 823, 743, 703; M.p 166–170 °C. **8e**, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.86 (d, 1H, C*H*=CH, J = 16.0 Hz), 7.69 (m, 1H, aryl-H), 7.45 (d, 2H, aryl-H, J = 8.4 Hz), 7.34 (m, 1H, aryl-H), 7.30 (d, 2H, aryl-H, J = 8.4 Hz), 7.21 (m, 2H, aryl-H), 7.07 (d, 1H, CH=C*H*, J = 16.0 Hz), 5.07 (s, 2H, Fc-CH_2), 4.16 (m, 2H, cpd-H), 4.08 (s, 5H, cpd-H), 4.04 (m, 2H, cpd-H); ^{13}C (100 MHz, $CDCl_3$) δ (ppm) 150.5, 143.5, 136.0, 135.7, 135.2, 135.0, 129.6, 128.8, 123.2, 123.1, 119.9, 114.3, 109.9, 83.9, 69.3, 68.9, 68.8, 43.4; IR (KBr) ν (cm^{-1}) 3051, 2971, 2320, 1718, 1632, 1500, 1454, 1420, 1386, 1334, 1265, 1099, 1013, 967, 892, 818, 737, 703, 639; mp 188–192 °C.

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