Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 3799–3802
Bioorganic & Medicinal Chemistry Letters

Synthesis and structure–activity relationship of novel benzoxazole derivatives as melatonin receptor agonists

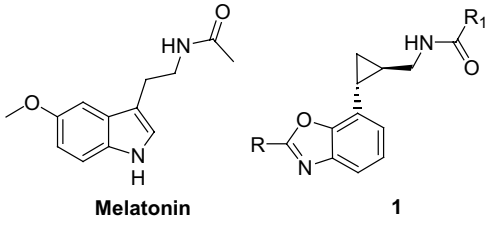
Li-Qiang Sun,* Jie Chen, Marc Bruce,† Jeffrey A. Deskus, James R. Epperson, Katherine Takaki, Graham Johnson,‡ Lawrence Iben, Cathy D. Mahle,§ Elaine Ryan and Cen Xu

Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492, USA

Received 23 March 2004; revised 26 April 2004; accepted 27 April 2004

Abstract—A series of benzoxazole derivatives was synthesized and evaluated as melatoninergic ligands. The binding affinity of these compounds for human MT_1 and MT_2 receptors was determined using 2-[^{125}I]-iodomelatonin as the radioligand. From this series of benzoxazole derivatives, compounds **14** and **17** were identified as melatonin receptor agonists.
© 2004 Elsevier Ltd. All rights reserved.

The hormone melatonin (*N*-acetyl-5-methoxy-tryptamine) (Fig. 1) is synthesized and released primarily by the pineal gland in a circadian manner that closely follows the daily light/dark cycle.[1,2] It plays a major role in the regulation of circadian rhythms and the control of seasonal cycles.[3,4] Melatonin alleviates jet lag, regulates delayed sleep phase syndrome,[5] and induces sleep.[6] It has been demonstrated that many of the effects of melatonin are mediated through G-protein-coupled receptors expressed primarily in the brain, retina, pituitary, and blood vessels.[7] The recent cloning of several G-protein-coupled melatonin receptor genes has revealed at least three melatonin receptor subtypes, two of which are defined as MT_1 and MT_2 and have been found in mammals.[8] The human MT_1 and MT_2 receptor subtypes show 55% overall identity at the amino acid level and about 70% identity within the transmembrane domains where ligand binding is thought to occur. Both MT_1 and MT_2 receptors are negatively coupled to adenylate cyclase and exhibit subnanomolar binding affinity for melatonin.[9,10] While it is known that the MT_1 and MT_2 receptors are expressed both centrally and peripherally, the physiological roles of these receptors are not as well defined. A few MT_2 selective ligands have been described to date.[11–18] Several physiological responses mediated through the activation of MT_2 receptor have been identified using some of these compounds.[19] This receptor appears to play the major role in mammalian circadian entrainment since in mice lacking the MT_1 receptor, melatonin retains phase shifting activity that can be blocked by a MT_2-selective antagonist.[20] However, there are only two examples of MT_1 selective ligands provided in the literature until last year,[17,21] the lack of which has made it difficult to discern the physiological role of the subtype. Therefore, a need exists for ligands that demonstrate selectivity at the receptor level and particularly for MT_1. Such ligands would be valuable tools to help define the subtle



Melatonin 1

Figure 1.

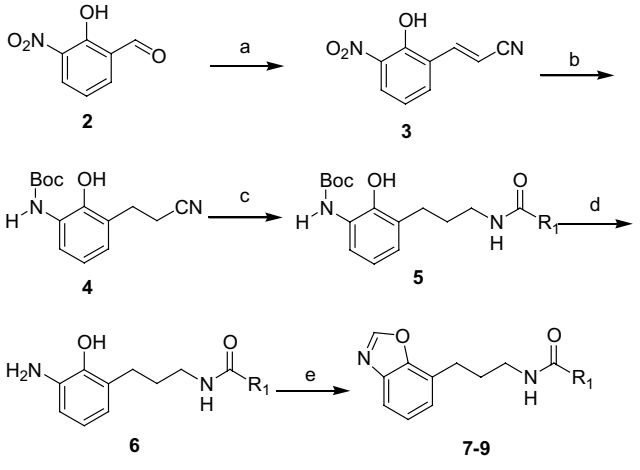
Keywords: Melatonin; Melatonin receptors; Agonists; Benzoxazole.
* Corresponding author. Tel.: +1-203-677-7460; fax: +1-203-677-7702; e-mail: sunl@bms.com
† Present address: Arena Pharmaceuticals, Inc., 6166 Nancy Ridge Drive, San Diego, CA 92121, USA.
‡ Present address: Rib-X Pharmaceuticals, Inc., 300 George Street, New Haven, CT 06511, USA.
§ Present address: Bayer Pharmaceutical Research Center, 400 Morgan Lane, West Haven, CT 06516, USA.

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.04.082

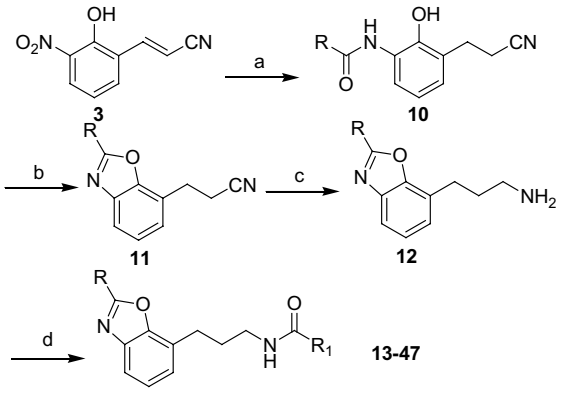
contributions of individual melatonin receptor subtypes to the multiple functions of melatonin, while also elucidating the pharmacological profile of subtype selective melatoninergic agents.

We have previously reported the discovery and binding characteristics toward human MT_1 and MT_2 melatonin receptors of a class of N-(2-benzoxazol-7-yl-cyclopropylmethyl)-amides such as **1** in Figure 1.[22] These melatoninergic ligands possess high binding affinity toward human MT_1 and MT_2 receptor subtypes, comparable to or higher than the affinity of melatonin itself. Of particular interest, these ligands bind to MT_1 receptor at the subnanomolar level and exhibit up to a 14-fold selectivity ratio over the MT_2 subtype. These results highlighted the utility of introducing conformational restraint in the alkyl tether that links the benzoxazole core with a terminal amide functionality as a means of modulating receptor affinity and selectivity. The earlier work represented part of a broader initiative to understand the SAR associated with benzoxazole-based melatoninergic agents in the context of MT_1 and MT_2 binding properties. In this article, we present a detailed description of the SAR associated with the two termini of this parent acylic benzoxazole chemotype. More specifically, the synthesis and biological activity of a new series of benzoxazole derivatives as novel and potent melatoninergic ligands are discussed. These studies incorporate a propyl amide motif that was shown to be optimal in a series of phenylalkyl amide derivatives.[23]

The synthetic routes utilized to procure the compounds of interest are shown in Schemes 1 and 2. These routes differ in that in Scheme 1 the oxazole ring is introduced after derivatization of the acyclic amine to its final form. This order for the synthetic transformations was found to be essential because the unsubstituted-oxazole ring system present in compounds **7**–**9** is unstable toward the conditions required for amine formation and derivatization. In Scheme 2, the order of these synthetic processes is reversed.



Scheme 1. Reagents and conditions: (a) $(EtO)_2POCH_2CN$, NaH, THF, 0 °C, 95%; (b) Boc_2O, 10% Pd/C, H_2, THF, 94%; (c) $(RCO)_2O$, Raney Ni, H_2, THF; (d) 4 N HCl, EtOAc, 40 °C; (e) $CH(OEt)_3$, PPTS, xylene, reflux.



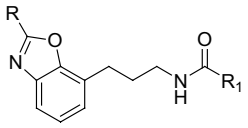
Scheme 2. Reagents and conditions: (a) $(RCO)_2O$, 10% Pd/C, H_2, THF; (b) PPTS, xylene, reflux; (c) Raney Ni, H_2, MeOH/NH_4OH; (d) RCOCl, Et_3N, CH_2Cl_2 or RNCO, benzene.

As outlined in Scheme 1, the synthesis of the designed compounds **7**–**9** started from commercially available 3-nitrosalicylaldehyde **2**, which was homologated with diethylcyanomethylphosphonate using a Horner–Wadsworth–Emmons reaction to afford nitrile **3** in excellent (95%) yield. Cinnamonitrile **3** was converted to carbamate **4** in a one-pot procedure wherein **3** was hydrogenated over 10% Pd/C in the presence of $(Boc)_2O$. Further reduction of nitrile **4** with Raney Nickel in the presence of the appropriate anhydride yielded a series of amides **5**. Removal of the Boc group, using hydrochloric acid, produced aniline **6**, which was closed to the benzoxazole heterocycle using triethyl *ortho*-formate in the presence of a catalytic amount of PPTS under reflux to provide target compounds **7**–**9**.

The general route to the 2-substituted benzoxazole derivatives **13**–**47** is described in Scheme 2. In brief, the nitrile **3** was hydrogenated over 10% Pd/C in the presence of an anhydride followed by quenching of this reaction with aqueous sodium hydroxide to afford amide **10**. Heating **10** with a catalytic amount of PPTS effected dehydration to give the benzoxazole **11**. Hydrogenation of the nitrile moiety of **11** with Raney Nickel afforded the primary amine **12**, which was derivatized with an acid chloride or an isocyanate to give the desired amide and urea products, respectively. These compounds, **13**–**47**, are compiled in Table 1.

The K_i values of compounds **7**–**9** and **13**–**47** for human MT_1 and MT_2 melatonin receptor subtypes were determined in assays using 2-[^{125}I]-iodomelatonin as the ligand according to described methods.[24] The chemical structures, K_i values and MT_2/MT_1 selectivity ratios of these compounds are reported in Table 1. Compared to melatonin, the benzoxazoles **7**–**9**, in which there is a hydrogen at the 2-position of the benzoxazole ring (R = H), demonstrated high affinity for both MT_1 and MT_2 receptors. On the other hand, these ligands showed a slight change in binding affinity ongoing from acetamide **7** to butyramide **9** with the exception that

Table 1. Binding affinity of compounds **7**–**9** and **13**–**47** for human MT_1 and MT_2 melatonin receptors stably expressed in NIH3T3 cells. Values represent mean from experiments performed in duplicate



7-9 and **13-47**

Compd	R	R_1	MT_1 K_i (nM)	MT_2 K_i (nM)	Ratio MT_2/MT_1
Mel	—	—	0.4	0.3	0.75
7	H	Me	30	4.3	0.14
8	H	Et	6.6	1.8	0.27
9	H	*n*-Pr	9.9	3.7	0.37
13	Me	Me	24	5.3	0.22
14	Me	Et	2.8	2.8	1.0
15	Me	*n*-Pr	2.3	2.2	0.96
16	Me	*i*-Pr	3.0	2.3	0.8
17	Me	*c*-Pr	0.7	2.7	3.8
18	Me	NHEt	50	26	0.52
19	Et	Me	9.0	2.3	0.25
20	Et	Et	3.5	1.0	0.28
21	Et	*n*-Pr	8.1	1.0	0.12
22	Et	*i*-Pr	13.2	4.5	0.34
23	Et	*c*-Pr	4.5	4.7	1.0
24	Et	NHEt	75	12	0.16
25	*n*-Pr	Me	183	35	0.19
26	*n*-Pr	Et	51	27	0.53
27	*n*-Pr	*n*-Pr	166	22	0.13
28	*n*-Pr	*i*-Pr	60	29	0.48
29	*n*-Pr	*c*-Pr	44	7.6	0.17
30	*n*-Pr	NHEt	492	36	0.07
31	*i*-Pr	Me	116	35	0.30
32	*i*-Pr	Et	142	15	0.11
33	*i*-Pr	*n*-Pr	119	16	0.13
34	*i*-Pr	*i*-Pr	40	88	2.2
35	*i*-Pr	*c*-Pr	34	114	3.3
36	*i*-Pr	NHEt	194	52	0.27
37	Ph	Me	40	89	2.2
38	Ph	Et	13.1	10	0.77
39	Ph	*n*-Pr	60	17	0.28
40	Ph	*i*-Pr	87	57	0.66
41	Ph	*c*-Pr	23	38	1.6
42	Ph	NHEt	340	61	0.18
43	$Ph(CH_2)_4$	Me	1.3	16	12
44	$Ph(CH_2)_4$	Et	0.76	14	18
45	$Ph(CH_2)_4$	*n*-Pr	3.3	29	9
46	$Ph(CH_2)_4$	*i*-Pr	1.3	18	14
47	$Ph(CH_2)_4$	*c*-Pr	0.63	22	35

Data reported in the text are means of 1–2 experiments run at five different concentrations in duplicates. Standard errors were typically within 10% of mean value. Melatonin was run as standard reference in every assay with reproducible K_i.

acetamide **7** exhibited relatively weaker MT_1 binding. This series demonstrated poor to modest selectivity for the MT_2 receptor with a ratio of MT_1/MT_2 ranging from 2 to 7.

The introduction of a methyl group at the 2-position of the benzoxazole heterocycle provided a series of ligands **13**–**18** from which the parent compound **13** is a potent MT_2 ligand that exhibits fourfold weaker MT_1 affinity, providing a modest index of receptor selectivity. Homologation of the methyl group of the amide side chain to ethyl (**14**), propyl (**15**), or *iso*-propyl (**16**) resulted in a higher increase in MT_1 affinity compared to MT_2 receptor binding, leading to a reduction in receptor selectivity. Interestingly, pursuing a similar substitution pattern with the cyclopropyl derivative **17** resulted in the achievement of subnanomolar MT_1 affinity that was accompanied by a twofold increase in MT_2 binding. Moreover, **17** showed little selectivity toward the MT_1 receptor subtype (ratio = 3.8). Replacing the terminal amide of **17** with a simple ethyl urea provided a compound, **18**, which demonstrated a marked reduction in binding affinity at both receptors. Compounds **19**–**24**, in which R is an ethyl moiety instead of a methyl group, showed a trend toward increased MT_2 affinity and reduced MT_1 binding. Of this series, only the amide **21** and urea **24** exhibited modest selectivity for the MT_2 receptor subtype. However, the introduction of an *n*-propyl, *iso*-propyl, or a phenyl group at the 2-position of the benzoxazole ring, compounds **25**–**42**, resulted in a significant decrease in binding affinity toward both human MT_1 and MT_2 receptors while retaining the same limited level of subtype selectivity as melatonin itself. Increased selectivity for the MT_1 subtype was observed when a 4-phenylbutyl group was introduced at the benzoxazole 2-position, as exemplified by compounds **43**–**47**. This series demonstrates excellent affinity for the MT_1 subtype and relatively modest binding affinity for the MT_2 receptor. Consequently these analogues show MT_2/MT_1 selectivity ratios ranging from 9 to 35 with compound **47** the most selective ligand identified in this study.

The two most attractive compounds emerging from this series, **14** and **17**, were further tested for their functional activity in NIH3T3 cells expressing melatonin MT_1 or MT_2 receptor.[25] The agonist activity of these compounds was assessed by comparing their ability to inhibit forskolin-stimulated cAMP accumulation with that of melatonin. Full agonist activity was confirmed for both compounds at both the human melatonin MT_1 and MT_2 receptor subtypes. At the MT_1 receptor the EC_{50}s for compounds **14** and **17** were measured as 0.26 and 0.17 nM, respectively, while the relative intrinsic activity of these two analogues was 1.1 and 1.0, respectively. At MT_2 receptor, the EC_{50} values obtained for benzoxazoles **14** and **17** were 0.32 and 0.74 nM, respectively, with intrinsic activities of 0.98 and 0.96 compared to that of melatonin, respectively.

In conclusion, the constrained cyclopropane moiety of N-(2-benzoxazol-7-yl-cyclopropylmethyl)amide was successfully replaced by a conformationally flexible alkyl side chain. This structural modification led to the discovery of a series of benzoxazole derivatives[26] as novel melatoninergic ligands and the subsequent identification of benzoxazole derivative **14** and **17** as melatonin receptor agonists and compound **47** as a highly potent human MT_1 ligand with moderate receptor selectivity. Structure–activity relationships have been defined around both the terminal amide moiety and the C2 position of the benzoxazole ring. Furthermore, a 4-phenybutyl moiety at the 2-position of the

benzoxazole heterocycle provides compounds with enhanced MT_1 receptor activity and selectivity.

Acknowledgements

We thank Ms. Yi-Xin Li for NMR data support, Mr. Robert Kane for MS data support, and Dr. Nicholas A. Meanwell for valuable discussion.

References and notes

1. Yu, H.-S.; Reiter, R. J. Melatonin. *Biosynthesis, Physiological Effects, and Clinic Applications*; CRC Press: Boca Raton, 1993.
2. Arendt, J. *Melatonin and the Mammalian Pineal Gland*; Chapman and Hall: London, 1995.
3. Cassone, V. M. *Trends Neurosci.* **1990**, *13*, 457.
4. Bartness, T. J.; Goldman, B. D. *Experientia* **1989**, *45*, 939.
5. Arendt, J.; Deacon, S.; English, J.; Hampton, S.; Morgan, L. *J. Sleep Res.* **1995**, *4*, 74.
6. Wirz-Justice, A.; Amstrong, S. M. *J. Sleep Res.* **1996**, *5*, 137.
7. Morgan, P. J.; Barrett, P.; Howell, H. E.; Helliwell, R. *Neurochem. Int.* **1994**, *24*, 101.
8. Reppert, S. M.; Weaver, D. R.; Godson, C. *Trends Pharmacol. Sci.* **1996**, *17*, 100.
9. Reppert, S. M.; Weaver, D. R.; Ebisawa, T. *Neuron* **1994**, *13*, 1177.
10. Reppert, S. M.; Godson, C.; Mahle, C. D.; Weaver, D. R.; Slaugenhaupt, S. A.; Gusella, J. F. *Proc. Natl. Acad. Sci.* **1995**, *92*, 8734.
11. Teh, M.-T.; Sugden, D. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1998**, *358*, 522.
12. Faust, R.; Garratt, P. J.; Jones, R.; Yeh, L.-K.; Tsotinis, A.; Panoussopoulou, M.; Calogeropoulou, T.; Teh, M.-T.; Sugden, D. *J. Med. Chem.* **2000**, *43*, 1050.
13. Spadoni, G.; Balsamini, C.; Diamantini, G.; Tontini, A.; Tarzia, G.; Mor, M.; Rivara, S.; Plazzi, P. V.; Nonno, R.; Lucini, V.; Pannacci, M.; Fraschini, F.; Stankov, B. M. *J. Med. Chem.* **2001**, *44*, 2900.
14. Wallez, V.; Durieux-Poissonnier, S.; Chavatte, P.; Boutin, J. A.; Audinot, V.; Nicolas, J.-P.; Bennejean, C.; Delagrange, P.; Renard, P.; Lesieur, D. *J. Med. Chem.* **2002**, *45*, 2788.
15. Mattson, R. J.; Catt, J. D.; Keavy, D.; Sloan, C. P.; Epperson, J.; Gao, Q.; Hodges, D. B.; Iben, L.; Mahle, C. D.; Ryan, E.; Yocca, F. D. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1199.
16. Yous, S.; Durieux-Poissonnier, S.; Lipka-Belloli, E.; Guelzim, H.; Bochu, C.; Audinot, V.; Boutin, J. A.; Delagrange, P.; Bennejean, C.; Renard, P.; Lesieur, D. *Bioorg. Med. Chem.* **2003**, *11*, 753.
17. Audinot, V.; Mailliet, F.; Lahaye-Brasseur, C.; Bonnaud, A.; Gall, A. L.; Amosse, C.; Dromaint, S.; Rodriguez, M.; Nagel, N.; Galizzi, J.-P.; Malpaux, B.; Guillaumet, G.; Lesieur, D.; Lefoulon, F.; Renard, P.; Delagrange, P.; Boutin, J. A. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2003**, *367*, 553.
18. Epperson, J. R.; Deskus, J. A.; Gentile, A. J.; Iben, L. G.; Ryan, E.; Sarbin, N. S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1023.
19. Masana, M. I.; Dubocovich, M. L. *Sci. STKE* **2001**, *6*, 107.
20. Dubocovich, M. L.; Yun, K.; Al-Ghoul, W. M.; Benloucif, S.; Masana, M. I. *FASEB J.* **1998**, *12*, 1211.
21. Descamps-Francois, C.; Yous, S.; Chavatte, P.; Audinot, V.; Bonnaud, A.; Boutin, J. A.; Delagrange, P.; Bennejean, C.; Renard, P.; Lesieur, D. *J. Med. Chem.* **2003**, *46*, 1127.
22. Sun, L.-Q.; Chen, J.; Takaki, K.; Johnson, G.; Iben, L.; Mahle, C. D.; Ryan, E.; Xu, C. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1197.
23. Garratt, P. J.; Travard, S.; Vonhoff, S. *J. Med. Chem.* **1996**, *39*, 1797.
24. Sun, L.-Q.; Chen, J.; Mattson, R. J.; Epperson, J. R.; Deskus, J. A.; Li, W.-S.; Takaki, K.; Hodges, D. B.; Iben, L.; Mahle, C. D.; Ortiz, A.; Molstad, D.; Ryan, E.; Yeleswaram, K.; Xu, C.; Luo, G. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4381.
25. Mattson, R. J.; Catt, J. D.; Keavy, D.; Sloan, C. P.; Epperson, J.; Gao, Q.; Hodges, D. B.; Iben, L.; Mahle, C. D.; Ryan, E.; Yocca, F. D. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1199.
26. Selected spectral data. Compound **3**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.20 (dd, $J = 8.4$, 1.6 Hz, 1H), 7.73–7.70 (m, 1H), 7.64 (d, $J = 16.8$ Hz, 1H), 7.08 (t, $J = 7.7$ Hz, 1H), 6.26 (d, $J = 16.8$ Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.8, 143.7, 136.4, 134.3, 127.3, 125.0, 120.1, 118.0, 100.6; MS (ESI) 189 $(M-H)^+$. Compound **10**: (N-[3-((1E)-2-cyanoethyl)-2-hydroxyphenyl]-5-phenylpentanamide): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 (s, 1H), 7.54–7.06 (m, 6H), 6.91–6.72 (m, 2H), 3.03 (t, $J = 7.3$ Hz, 2H), 2.80–2.65 (m, 4H), 2.48 (t, $J = 5.5$ Hz, 2H), 1.84–1.67 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.0, 147.7, 142.0, 129.3, 128.6, 128.5, 126.1, 126.0, 125.8, 121.8, 120.3, 120.0, 36.8, 35.7, 30.9, 27.7, 25.5, 17.5; MS (ESI) 321 $(M-H)^+$. Compound **11**: (3-[2-(4-phenylbutyl)benzoxazol-7-yl]propanenitrile): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62 (d, $J = 7.8$ Hz, 1H), 7.31–7.06 (m, 7H), 3.26 (t, $J = 7.5$ Hz, 2H), 3.02 (t, $J = 7.3$ Hz, 2H), 2.94–2.78 (m, 4H), 1.96–1.74 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.2, 149.2, 142.0, 141.3, 128.6, 128.5, 126.0, 124.9, 124.8, 121.1, 119.0, 118.8, 35.6, 31.0, 28.6, 26.5, 26.4, 17.9; MS (ESI) 305 $(M+H)^+$. Compound **20**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (dd, $J = 7.8$, 0.9 Hz, 1H), 7.18 (t, $J = 7.6$ Hz, 1H), 7.05 (d, $J = 7.3$ Hz, 1H), 5.56 (br s, 1H), 3.41 (q, $J = 6.9$ Hz, 2H), 2.95–2.81 (m, 4H), 2.15 (q, $J = 7.6$ Hz, 2H), 1.94–1.80 (m, 2H), 1.41 (t, $J = 7.5$ Hz, 3H), 1.04 (t, $J = 7.6$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.8, 168.0, 149.5, 140.7, 124.7, 124.6, 124.4, 117.3, 39.1, 29.8, 29.7, 27.4, 22.2, 11.0, 10.0; MS (ESI) 259 $(M-1)^+$.