Bioorganic & Medicinal Chemistry Letters 22 (2012) 3269–3273

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

New diarylureas and diarylamides possessing acet(benz)amidophenyl scaffold: Design, synthesis, and antiproliferative activity against melanoma cell line

Hee Jin Kim [a], Hye Jung Cho [a], Hwan Kim [a], Mohammed I. El-Gamal [b,c], Chang-Hyun Oh [b,c], So Ha Lee [a], Taebo Sim [a], Jung-Mi Hah [d], Kyung Ho Yoo [a,*]

[a] Chemical Kinomics Research Center, Korea Institute of Science and Technology, PO Box 131, Cheongryang, Seoul 130-650, Republic of Korea
[b] Center for Biomaterials, Korea Institute of Science and Technology, PO Box 131, Cheongryang, Seoul 130-650, Republic of Korea
[c] Department of Biomolecular Science, University of Science and Technology, 113 Gwahangno, Yuseong-gu, Daejeon 305-333, Republic of Korea
[d] Department of Pharmacy, College of Pharmacy, Hanyang University 1271 Sa 3-dong, Sangnok-gu, Ansan-si, Gyunggi-do 426-791, Republic of Korea

ARTICLE INFO

Article history:
Received 20 January 2012
Revised 21 February 2012
Accepted 6 March 2012
Available online 11 March 2012

Keywords:
Melanoma
Antiproliferative activity
Acet(benz)amidophenyl
Diarylurea
Diarylamide
A375P

ABSTRACT

A series of new diarylurea and diarylamide derivatives possessing acet(benz)amidophenyl scaffold was synthesized. Their in vitro antiproliferative activity was tested against A375P human melanoma cell line. Compounds **1c,d** and **2c,d** showed the highest potencies with IC_{50} values in sub-micromolar scale. In addition, compounds **1b,e,l** and **2e,l** were more potent than Sorafenib but with IC_{50} values in micromolar range. Moreover, compound **2c** was equipotent to Vemurafenib, and **2d** showed higher potency than Vemurafenib against A375P. Molar refractometry calculation and ADME profiling of the highest potent four derivatives **1c,d** and **2c,d** are also reported.

Melanoma is a malignant tumor that arises from melanocytic cells and primarily involves the skin. Exposure to solar ultraviolet irradiation, fair skin, dysplastic nevi syndrome, and a family history of melanoma are major risk factors for melanoma development. Melanomas can metastasize either by the lymphatic or by the hematogenous route.[1] Metastatic melanoma is a particularly aggressive form of cancer that is resistant to standard anticancer therapies. Early stage melanoma (stage I/II) primary tumors can be surgically resected with more than 95% success rate.[2] In contrast, late-stage (stage IV) metastatic melanoma is one of the most deadly forms of cancer, with the median survival of patients with distant metastases being 7–8 months.[3] With the rapid incidence of melanoma in the United States and other developed countries, there is an urgent need to develop more effective drugs.[4–6]

In 2011, Vemurafenib (PLX4032, Zelboraf®) was approved by the US food and drug administration (FDA) for treatment of late-stage melanoma.[7] In addition, a number of reports have recently highlighted diarylureas and diarylamides as potential antiproliferative agents against melanoma cell lines.[8–17] Sorafenib (Nexavar®) is a diarylurea derivative that has been extensively used in clinical trials.[18] Encouraged by the interesting antiproliferative activity of diarylurea and diarylamide derivatives, we synthesized a new series of diarylureas and diarylamides containing acet(benz)amidophenyl scaffold (Fig. 1). Their in vitro antiproliferative activity against A375P human melanoma cell line is reported. In addition, molar refractometry and ADME predictions of the most potent target compounds are also reported.

The target compounds **1–4** were synthesized according to the sequence of reactions illustrated in Scheme 1. Heating a solution of 2-methyl-5-nitrobenzenamine (**5**) in acetic anhydride afforded the acetamido intermediate **6**.[19] Treatment of **5** with benzoyl chloride gave the benzamido compound **7**. Reduction of the nitro group of **6, 7** using palladium over carbon in hydrogen atmosphere produced the corresponding amino derivatives **8, 9** in good yields. Interaction of the amino groups of **8, 9** with the appropriate aryl isocyanate led to formation of the target diarylurea derivatives **1a–l** and **2a–l**, respectively. Synthesis of the diarylamides **3a–e** and **4a–e** was carried out by condensation of the amino groups of **8, 9** with the appropriate carboxylic acid derivatives in the presence of HOBt, EDCI, and triethylamine.

* Corresponding author. Address: Biomolecular Functional Research Center, Korea Institute of Science and Technology, PO Box 131, Cheongryang, Seoul 130-650, Republic of Korea. Tel.: +82 2 958 5152; fax: +82 2 958 5189.
E-mail address: khyoo@kist.re.kr (K.H. Yoo).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.03.020

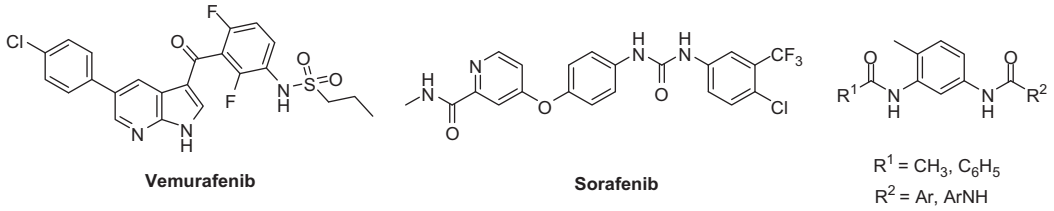
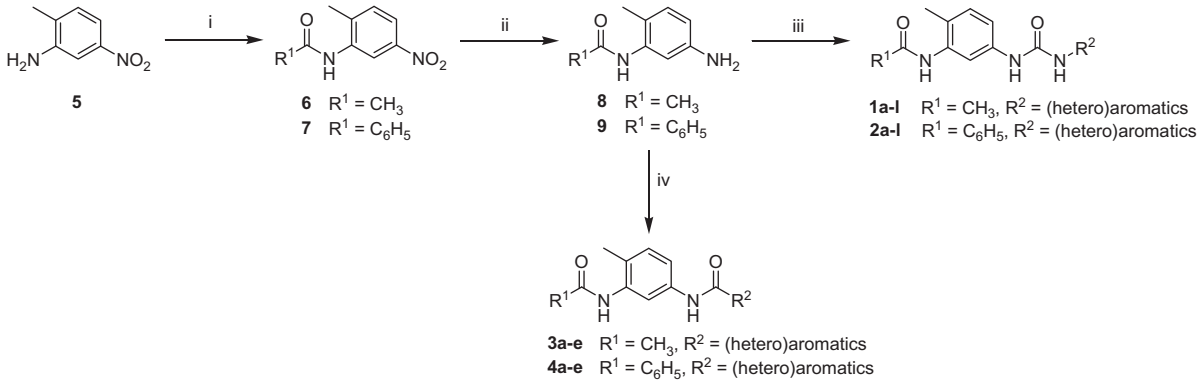


Figure 1. Structures of Vemurafenib, Sorafenib, and the target compounds.



Scheme 1. Reagents and conditions: (i) $(CH_3CO)_2O$, 90 °C, 3 h, 78% (for **6**), C_6H_5COCl, CH_2Cl_2, rt, 3 h, 69% (for **7**); (ii) Pd/C, H_2, MeOH, rt, 2 h, 93% (for **8**), 85% (for **9**); (iii) R^2-NCO, THF, rt, 4 h, 5–76% (for **1a–l**), 54–64% (for **2a–l**); (iv) R^2-CO_2H, HOBt, EDCI, Et_3N, DMF, 90 °C, 12 h, 22–73% (for **3a–e**), 25–64% (for **4a–e**).

The antiproliferative activity of the newly synthesized compounds against A375P human melanoma cell line was tested. The ability of acet(benz)amidophenylurea and diarylamide derivatives to inhibit the growth of A375P cell line is summarized in Tables 1 and 2. Sorafenib was selected as a reference standard because it has been extensively used in clinical trials for treatment of melanoma.[4,20] Vemurafenib was also utilized as a second reference standard in this experiment because of its high potency against melanoma cell lines,[21] and it has been recently approved by the FDA for treatment of advanced melanoma.[7]

Compounds **1d,e** and **2d,e** with urea linker were more potent than the corresponding compounds **3a,b** and **4a,b** with amide group as a linker. In general, the urea compounds in Table 1 demonstrated higher potencies than compounds in Table 2 with amide linker. This may be attributed to that the longer spacer, urea moiety, may geometrically permit appropriate fitting of the molecule at the receptor site. Or the terminal NH group of the urea moiety may form additional hydrogen bond(s) at the receptor site. Any or both of these effects would enable optimal drug–receptor interaction, and hence higher antiproliferative activity.

The effect of substituents of the terminal aryl ring on potency was also investigated. Compounds **1b** and **2b** with terminal 3,4-dichlorophenyl ring were more potent than **1g** and **2g** with 3,4-dimethylphenyl moiety. So we may conclude that electron-withdrawing groups on the terminal ring are more favorable for activity than electron-donating groups. In addition, 3,5-dichlorophenyl derivatives **1c** and **2c** showed higher potencies than **1b** and **2b** with 3,4-dichlorophenyl ring. This may be attributed to different directions of the terminal groups at the receptor site, which may affect the drug-receptor interaction. Moreover, compounds **1d** and **2d** with 3,5-bis(trifluoromethyl)phenyl, in addition to **1e** and **2e** with 4-chloro-3-(trifluoromethyl)phenyl ring were more potent than **1f** and **2f** with 3-fluoro-5-(trifluoromethyl)phenyl ring. This indicates that fluoro substituent on the terminal ring is unfavorable for activity.

Among all the target compounds, it was found that urea compounds **1c** and **2c** with 3,5-dichlorophenyl terminal ring, and **1d** and **2d** with 3,5-bis(trifluoromethyl)phenyl ring are the most potent derivatives of this series, with IC_{50} values in sub-micromolar range. So it can be concluded that these terminal rings together with urea spacer are optimum for antiproliferative activity of this series of compounds against melanoma cells. In addition, benzamido derivatives **2c** and **2d** were found to be more potent than acetamido compounds **1c** and **1d**. This may be due to hydrophobic interaction and/or electronic differences between benzamido phenyl ring and acetamido methyl group, which may affect the drug–receptor interaction.

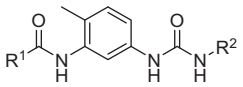
Compounds **1b** (with 3,4-dichlorophenyl terminal ring), **1e, 2e** (with 4-chloro-3-(trifluoromethyl)phenyl ring), and **1l, 2l** (with chloropyridyl ring) were more potent than Sorafenib but with IC_{50} values in micromolar scale. These terminal aryl moieties together with 3,5-dichlorophenyl and 3,5-bis(trifluoromethyl)phenyl are the optimal moieties for this series of compounds.

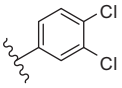
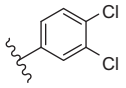
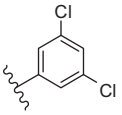
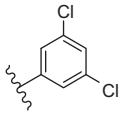
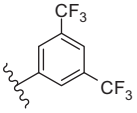
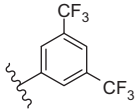
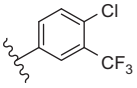
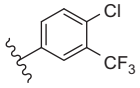
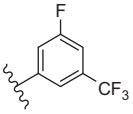
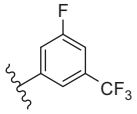
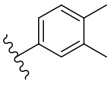
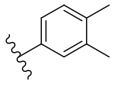
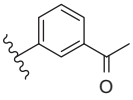
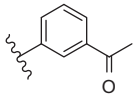
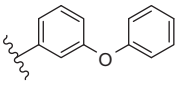
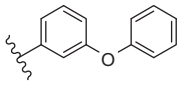
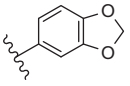
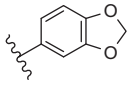
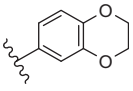
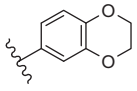
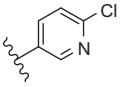
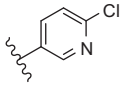
As compared with Vemurafenib, compound **2c** demonstrated the same potency. And compound **2d** showed 1.57 times higher potency against A375P than Vemurafenib.

The influence of steric factors on the antiproliferative activity was investigated. Molar refractometry (MR, steric parameter) values were determined for the compounds with the highest potencies **1c,d** and **2c,d**, and are presented in Table 3. The benzamido compounds **2c,d** with the highest MR values showed the highest potency over A375P melanoma cell lines. And the acetamido derivatives **1c,d** with lower bulkiness and MR values were less potent against A375P, compared with **2c,d**. From these results, we can conclude that MR and bulkiness are directly proportional to the antiproliferative activity of this series of compounds. The bulkier benzamido moiety may enable the appropriate fitting at the receptor site, and hence higher antiproliferative activity.

The bioavailability of compounds **1c,d** and **2c,d** was assessed using ADME (absorption, distribution, metabolism, and excretion) prediction methods. In particular, we calculated the compliance of compounds to the Lipinski's rule of five.[22] This approach has been widely used as a filter for substances that would likely be further developed in drug design programs. In addition, we calculated the total polar surface area (TPSA) since it is another key property that has been linked to drug bioavailability. Thus, passively ab-

Table 1
Antiproliferative activity of acetamidophenylureas **1a–l** and benzamidophenylureas **2a–l**

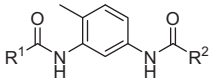


Compd No.	R^1	R^2	A375P (IC_{50}, μM)	Compd No.	R^1	R^2	A375P (IC_{50}, μM)
1a	CH_3		>30	**2a**	C_6H_5		>30
1b	CH_3		2.1	**2b**	C_6H_5		5.8
1c	CH_3		0.561	**2c**	C_6H_5		0.259
1d	CH_3		0.685	**2d**	C_6H_5		0.162
1e	CH_3		1.1	**2e**	C_6H_5		1.3
1f	CH_3		>30	**2f**	C_6H_5		>30
1g	CH_3		>30	**2g**	C_6H_5		>30
1h	CH_3		22.1	**2h**	C_6H_5		9.8
1i	CH_3		>30	**2i**	C_6H_5		>30
1j	CH_3		>30	**2j**	C_6H_5		>30
1k	CH_3		>30	**2k**	C_6H_5		>30
1l	CH_3		1.1	**2l**	C_6H_5		1.6
Sorafenib			2.7	Vemurafenib			0.254

sorbed molecules with a TPSA >140 are thought to have low oral bioavailability.[23] Molecules violating more than one of these rules may have problems with bioavailability. Predictions of ADME properties for the studied compounds are summarized in Table 3. The results showed that all the four tested compounds comply with these rules. Theoretically, compounds **1c,d** and **2c,d** should present good passive oral absorption and differences in their bioactivity cannot be attributed to this property.

In conclusion, a series of new acet(benz)amidophenylurea and bisamide derivatives was synthesized based on our previous literature studies, and as a continuation of our ongoing anticancer development program. Among all of these derivatives, compounds **1b–e**, **1l**, **2c–e**, and **2l** demonstrated higher potencies against A375P human melanoma cell line than that of Sorafenib. Of special interest, compounds **1c**[24] and **2c** possessing 3,5-dichlorophenylurea, in addition to **1d** and **2d**[24] with 3,5-bis(trifluoromethyl)phenylurea moiety showed the highest potencies with IC_{50} values in sub-micromolar scale. Among them, compound **2c** showed the same potency and **2d** was more potent, compared with Vemurafenib. Molar refractometry calculations showed that the increased bulkiness induced by benzamido moiety, in case of compounds **2c,d**, was favorable for activity. In silico ADME profiling showed that compounds **1c,d** and **2c,d** can be bioavailable through passive oral absorption. The superior potency of compound **2d** against

Table 2
Antiproliferative activity of the bisamides **3a–e** and **4a–e**



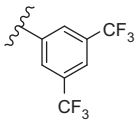
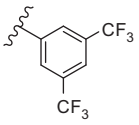
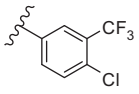
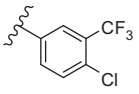
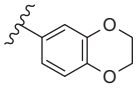
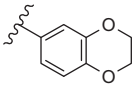
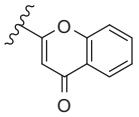
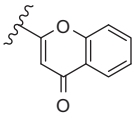
Compd No.	R^1	R^2	A375P (IC_{50}, μM)	Compd No.	R^1	R^2	A375P (IC_{50}, μM)
3a	CH_3		>30	**4a**	C_6H_5		>30
3b	CH_3		>30	**4b**	C_6H_5		>30
3c	CH_3		12.9	**4c**	C_6H_5		>30
3d	CH_3		>30	**4d**	C_6H_5		>30
3e	CH_3		>30	**4e**	C_6H_5		>30
Sorafenib			2.7	Vemurafenib			0.254

Table 3
Molar refractometry, solubility, and calculated Lipinski's rule of five for the most potent target compounds

Compd No.	IC_{50} (nM) over A375P cell line[a]	MR[b]	Log*S*[c]	Parameter					
				Log*P*[d]	TPSA[e]	MW[f]	*n*ON[g]	*n*OHNH[h]	*n*Violations
1c	561	89.72	−5.80	3.02	70.23	352.22	5	3	0
1d	685	93.52	−5.89	3.75	70.23	419.32	5	3	0
2c	259	109.84	−6.97	4.92	70.23	414.28	5	3	0
2d	162	113.64	−7.06	5.65	70.23	481.39	5	3	0

[a] Data taken from Table 1.
[b] Molar refractometry (cm^3/mol).
[c] Solubility parameter.
[d] Calculated lipophilicity.
[e] Total polar surface area ($Å^2$).
[f] Molecular weight.
[g] Number of hydrogen bond acceptors.
[h] Number of hydrogen bond donors.

A375P melanoma cell line to both Sorafenib and Vemurafenib, together with its in silico results make this compound a promising lead for development of new efficient and orally-bioavailable anticancer agents for treatment of melanoma.

Acknowledgments

This research was supported by Korea Institute of Science and Technology (KIST) and Fundamental R&D Program for Core Technology of Materials funded by the Ministry of Knowledge Economy, Republic of Korea (K0006028).

References and notes

1. Garbe, C.; Hauschild, A.; Volkenandt, M.; Schadendorf, D.; Stolz, W.; Reinhold, U.; Kortmann, R. D.; Kettelhack, C.; Frerich, B.; Keilholz, U.; Dummer, R.; Sebastian, G.; Tilgen, W.; Schuler, G.; Mackensen, A.; Kaufmann, R. *Melanoma Res.* **2007**, *17*, 393.
2. Balch, C. M.; Buzaid, A. C.; Soong, S. J.; Atkins, M. B.; Cascinelli, N.; Coit, D. G.; Fleming, I. D.; Gershenwald, J. E.; Houghton, A., Jr.; Kirkwood, J. M.; McMasters, K. M.; Mihm, M. F.; Morton, D. L.; Reintgen, D. S.; Ross, M. I.; Sober, A.; Thompson, J. A.; Thompson, J. F. *J. Clin. Oncol.* **2001**, *19*, 3635.
3. Lee, M. L.; Tomsu, K.; Von Eschen, K. B. *Melanoma Res.* **2000**, *10*, 81.
4. Gray-Schopfer, V.; Wellbrock, C.; Marais, R. *Nature* **2007**, *445*, 851.
5. Garbe, C.; Eigentler, T. K. *Melanoma Res.* **2007**, *17*, 117.
6. Koon, H. B.; Atkins, M. B. *Expert Rev. Anticancer Ther.* **2007**, *7*, 79.
7. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2011/ucm268241.htm.
8. Jung, M.-H.; Oh, C.-H. *Bull. Korean Chem. Soc.* **2008**, *29*, 2231.
9. Nam, B. S.; Kim, H.; Oh, C.-H.; Lee, S. H.; Cho, S. J.; Sim, T. B.; Hah, J.-M.; Kim, D. J.; Choi, J. H.; Yoo, K. H. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3517.
10. Jung, M.-H.; Kim, H.; Choi, W.-K.; El-Gamal, M. I.; Park, J.-H.; Yoo, K. H.; Sim, T. B.; Lee, S. H.; Baek, D.; Hah, J.-M.; Cho, J.-H.; Oh, C.-H. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6538.
11. Choi, W.-K.; Oh, C.-H. *Bull. Korean Chem. Soc.* **2009**, *30*, 2027.
12. Kim, H. J.; Jung, M.-H.; Kim, H.; El-Gamal, M. I.; Sim, T. B.; Lee, S. H.; Hong, J. H.; Hah, J.-M.; Cho, J.-H.; Choi, J. H.; Yoo, K. H.; Oh, C.-H. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 413.
13. Lee, J.; Kim, H.; Yu, H.; Chung, J. Y.; Oh, C.-H.; Yoo, K. H.; Sim, T.; Hah, J.-M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1573.

14. Yu, H.; Jung, Y.; Kim, H.; Lee, J.; Oh, C.-H.; Yoo, K. H.; Sim, T.; Hah, J.-M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3805.
15. Lee, J.; Nam, B. S.; Kim, H.; Oh, C.-H.; Lee, S. H.; Cho, S. J.; Sim, T. B.; Hah, J.-M.; Kim, D. J.; Tae, J.; Yoo, K. H. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5722.
16. El-Gamal, M. I.; Jung, M.-H.; Lee, W. S.; Sim, T.; Yoo, K. H.; Oh, C.-H. *Eur. J. Med. Chem.* **2011**, *46*, 3218.
17. Choi, W.-K.; El-Gamal, M. I.; Choi, H. S.; Baek, D.; Oh, C.-H. *Eur. J. Med. Chem.* **2011**, *46*, 5754.
18. Wilhelm, S. M.; Adnane, L.; Newell, P.; Villanueva, A.; Llovet, J. M.; Lynch, M. *Mol. Cancer Ther.* **2008**, *7*, 3129.
19. Glinsukon, T.; Weisburger, E. K.; Benjamin, T.; Roller, P. P. *J. Chem. Eng. Data* **1975**, *20*, 207.
20. Eisen, T.; Ahmad, T.; Flaherty, K. T.; Gore, M.; Kaye, S.; Marais, R.; Gibbens, I.; Hackett, S.; James, M.; Schuchter, L. M.; Nathanson, K. L.; Xia, C.; Simantov, R.; Schwartz, B.; Poulin-Costello, M.; O'Dwyer, P. J.; Ratain, M. J. *Br. J. Cancer* **2006**, *95*, 581.
21. Sala, E.; Mologni, L.; Truffa, S.; Gaetano, C.; Bollag, G. E.; Gambacorti-Passerini, C. *Mol. Cancer Res.* **2008**, *6*, 751.
22. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Delivery Rev.* **2001**, *46*, 3.
23. Clark, D. E.; Pickett, S. D. *Drug Discovery Today* **2000**, *5*, 49.
24. *Selected data*. Compound **1c**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.23 (s, 1H), 8.96 (s, 1H), 8.85 (s, 1H), 7.57 (d, J = 1.4 Hz, 1H), 7.54 (d, J = 1.8 Hz, 1H), 7.52 (d, J = 1.8 Hz, 2H), 7.19 (t, J = 1.8 Hz, 1H), 7.17 (d, 2.1 Hz, 1H), 7.14 (t, J = 1.8 Hz, 1H), 7.08 (d, J = 8.3 Hz, 1H), 2.13 (s, 3H), 2.05 (s, 3H); MS m/z 353 $(M+1)^+$. Compound **2d**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.60 (s, 1H), 9.30 (s, 1H), 8.60 (s, 1H), 8.36 (s, 1H), 8.20 (dd, J = 1.7, 8.0 Hz, 1H), 7.83 (s, 1H), 7.54 (dd, J = 2.7 Hz, 10.9, 2H), 7.32 (q, J = 7.3 Hz, 1H), 7.20 (d, J = 11.0 Hz, 1H), 7.15 (d, J = 7.3 Hz, 2H), 7.07 (q, J = 3.7 Hz, 2H), 2.30 (s, 3H); MS m/z 482 $(M+1)^+$.