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Synthesis and evaluation of amide side-chain modified Agomelatine analogues as potential antidepressant-like agents

Ying Chang [a,†], Weiyi Pi [a,†], Wei Ang [b], Yuanyuan Liu [a], Chunlong Li [c], Jiajia Zheng [c], Li Xiong [b], Tao Yang [a], Youfu Luo [a,*]

[a] National Key Laboratory of Biotherapy, West China Hospital, West China Medical School, Sichuan University, Chengdu, Sichuan 610041, PR China
[b] Key Laboratory of Drug Targeting and Drug Delivery System, Ministry of Education, West China School of Pharmacy, Sichuan University, Chengdu, Sichuan 610041, PR China
[c] Pharmaceutical and Biological Engineering Department, Institute for Chemical Engineering, Sichuan University, Chengdu, Sichuan 610041, PR China

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ABSTRACT

In this work, nineteen analogues of Agomelatine were readily synthesized through structural modification of the acetamide side-chain starting from the key common intermediate 2-(7-methoxynaphthalen-1-yl) ethanamine (**3**), which was prepared from commercially available compound 2-(7-methoxynaphthalen-1-yl) acetonitrile (**2**) in two steps. Corticosterone-induced PC12 pheochromocytoma cells phenotypic *in vitro* model was utilized to evaluate their potential antidepression activities. Imide compound **4a** and acylamino carboxylic acid analogue **5b** showed good protective effects on traumatic PC12 cells with protection rates of 34.2% and 23.2%, respectively. Further *in vivo* assessments in C57 mice FST (forced swim test) model demonstrated that compound **4a** significantly reduced the immobility time of the tested subjects, indicating antidepressant-like activity. Preliminary toxicity assays conducted on human normal liver L02 cells and embryonic kidney 293 cells suggested a relatively low safety risk for compound **4a** compared with the marketed drugs Agomelatine and Fluoxetine. The promising antidepressant-like efficacy of compound **4a**, together with the relatively low toxicity to the normal tested cells and high liability of diffusion through the blood–brain barrier (BBB), presents us insights of exploration of me-better drug candidates of Agomelatine.

Depression, with high prevalence worldwide, is a mental disorder, characterized by sadness, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, feelings of tiredness, and poor concentration.[1] By 2020, according to the WHO report, depression will be the second disease next to ischemic heart disease and become one of the major contributors to the global disease burden.[2]

The current antidepressant drugs in clinic can generally be classified into several categories, which include selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors (MAOIs), serotonin–norepinephrine reuptake inhibitors (SNRIs), noradrenaline reuptake inhibitors (NRIs), tricyclic antidepressants (TCAs) and dopamine–noradrenaline reuptake inhibitors (DNRIs) (Fig. 1).[3,4] Although these antidepressants are often helpful, their full efficacy is only apparent after several weeks of administration and many patients only partially respond, and some remain refractory and severe side effects.[5] Thus to search for new candidate agents with novel action mechanism is strongly desirable.

Agomelatine was approved for the treatment of major depression disorders by European Medicines Agency in 2009.[6] However, cases of liver injury, including hepatic failure,[7,8] elevations of liver enzymes exceeding 10 times the upper limit of normal, hepatitis and jaundice have been reported in patients treated with Agomelatine during the first months of the treatment. Although the serum transaminases usually returned to normal when discontinued use of Agomelatine, there is still a need for structural modification of Agomelatine in order to enhance its efficacy and decrease its toxicity.

In our continuing efforts to develop novel antidepression agents with improved pharmacological profiles than the marketed ones, we started a structural modification program of Agomelatine on its amide side-chain. Two structural classes of Agomelatine bioisomers, imides (Scheme 1) and asymmetrical ureas (Scheme 3), were synthesized and investigated for its potential effects on in vitro and in vivo depression models. The synthesis and biological assessment of the acylamino carboxylic acids (Scheme 2) were also undertook herein in consideration that they are ring-opening

* Corresponding author. Tel./fax: +86 28 85503817.
E-mail address: luo_youfu@scu.edu.cn (Y. Luo).
† Authors contributed to this work equally.

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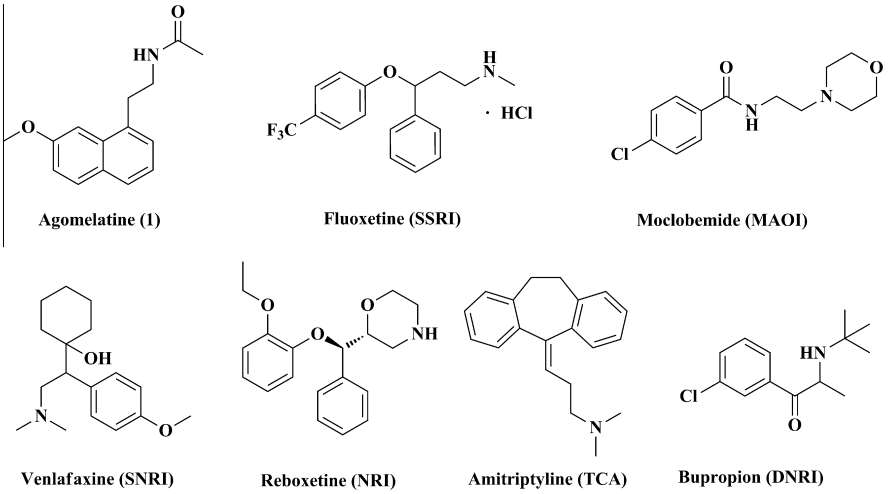
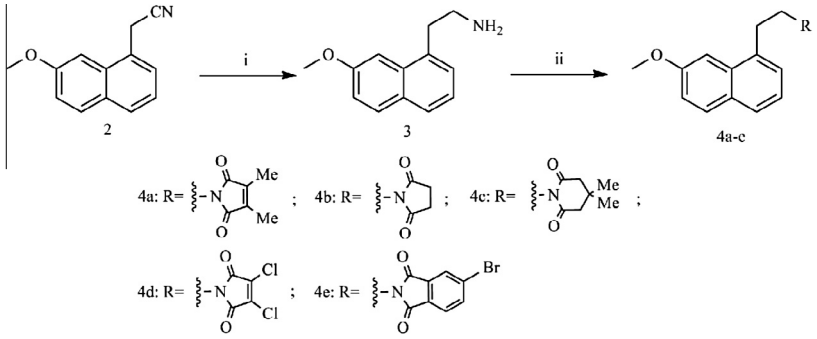
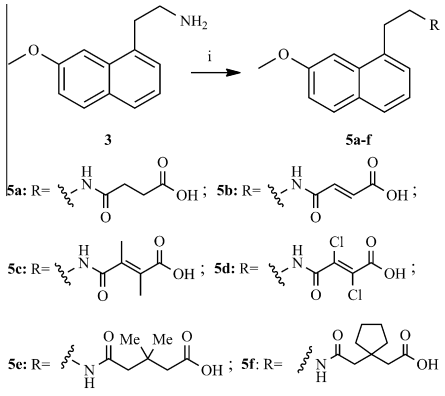


Figure 1. Chemical structures of representative antidepressants.



Scheme 1. Synthetic route of imide analogues **4a**–**e**. Reagents and conditions: (i) $NaBH_4$, $(Boc)_2O$, $NiCl_2$, MeOH; TFA, DCM; (ii) appropriate cyclic anhydride, NaOAc, HOAc, reflux, 3 h.



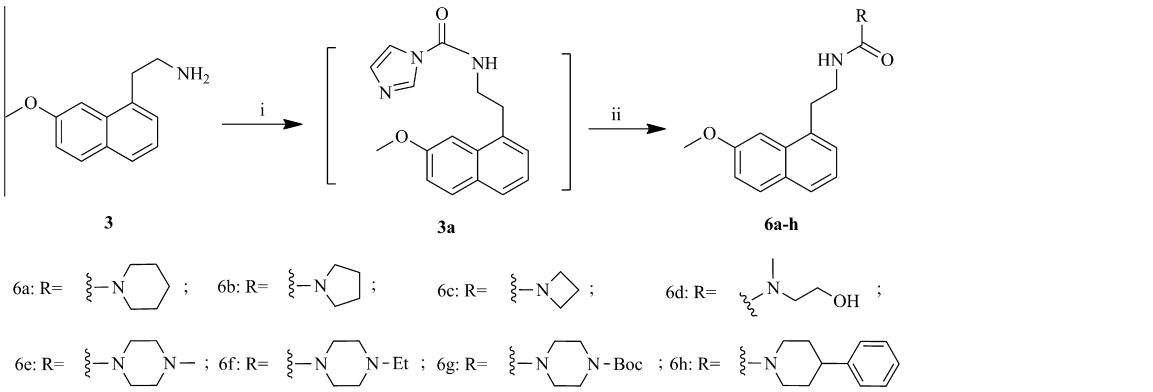
Scheme 2. Synthetic route of and acylamino carboxylic acid analogues **5a**–**f**. Reagents and conditions: (i) appropriate cyclic anhydride, CH_2Cl_2, rt, 6 h.

forms of the corresponding imides and easily prepared in similar reaction conditions, which can bring us insights when compared their effects with those of imides although acylamino carboxylic acid may not be a good candidate for passing the BBB for the high polarity of carboxylic acid group, which can be readily modified to its ester or carbamate form if needed.

To achieve the synthesis of the target compounds **4a**–**e**, the steps outlined in Scheme 1 were adopted. The key common intermediate **3** was synthesized starting from commercially available 2-(7-methoxynaphthalen-1-yl) acetonitrile in two steps according to literature[9] with minor revision. Firstly 2-(7-methoxynaphthalen-1-yl) acetonitrile was reduced to the corresponding amine with sodium borohydride/nickel chloride system followed by in situ N-Boc protection with dibutyldicarbonate in methanol. After N-Boc deprotection of compound **2** with trifluoroacetic acid in dichloromethane, the common key intermediate **3** was obtained. It is worthwhile to point out that the in situ N-Boc protection procedure is quite necessary, otherwise the direct reduction product would be contaminated with a mixture of side products such as oxime, secondary amine and acyl amine, which would turn into dark green when exposed to the atmosphere and was difficult to purify. Compounds **4**–**6** were prepared according to reported procedure.[10–13] The key intermediate **3** was reacted with the appropriate cyclic anhydride in the presence of sodium acetate and acetic acid under reflux for 3 h to obtained **4**.

The compounds **5a**–**f** can be prepared conveniently by stirring 2-(7-methoxynaphthalen-1-yl) ethanamine (**3**) with the appropriate cyclic anhydride in the presence of sodium acetate and acetic acid at room temperature for 6 h. For compounds **5a**–**e**, the amino group of compound **3** nucleophilic attack the carbon atom of carbonyl group in room temperature, formed target amide compounds. When heated to reflux, the secondary amino group of compounds **5a**–**f** further nucleophilic attack another carbon atom of carbonyl group, obtained **4a**–**e**.

The asymmetrical urea compounds (**6a**–**h**) were synthesized from the activated intermediate **3a** and appropriate secondary amine in the presence of triethyl amine at ambient condition. The non-isolated intermediate **3a** was made in situ by stirring



Scheme 3. Synthetic route of asymmetrical urea analogues of Agomelatine (**6a–h**). Reagents and conditions: (i) CDI, CH_3CN, DMF, 2 h; (ii) different substituted amine, Et_3N, 12 h.

the common key intermediate **3** and *N*,*N*′-carbonyldiimidazole (CDI) in acetonitrile and dimethylformamide at room temperature for 2 h.

The target compounds were determined to be 98% at least by HPLC–UV at full wavelength and were fully characterized by ^{1}H nuclear magnetic resonance (^{1}H NMR), ^{13}C NMR and mass spectroscopy (MS) before entering the biological tests.

In biological aspect, it is widely known that one of the bottlenecks to rapidly develop effective drugs for treatment of mental disorders including depression is poorly predictive capability of in vitro and in vivo screening models. Thus to develop or select suitable screening models is a major task which the researchers in this field have to confront with. In the mammalian brain, high levels of glucocorticoid receptors are expressed in hippocampus that controls emotional activity[13] and the excessive long-lasting plasma glucocorticoid-induced lesion in hippocampus may cause depression.[14–17] Research has shown that anti-glucocorticoid therapy in depressed patients is effective by renovation of the injured nerve cells, which was well illustrated by the widely used antidepressant Fluoxetine. PC12, derived from a pheochromocytoma of the rat adrenal medulla, is a cell line of easy subculture and storage.[18] The cellular morphology of PC12 cells would be partially affected if treated with suitable concentration of corticosterone, and some of which could be restored by the treatment of the known antidepressant such as Fluoxetine. The model of corticosterone-induced PC-12 lesion is widely used to test the antidepression activity of compounds.[4,14,19,20] As a result, evaluation of the protection effects on PC12 cells from corticosterone-induced lesion was an ideal phenotypic in vitro model for high-throughput screening of compounds with potential antidepression activity.[21] Therefore, we tested all the target compounds for their protection activities on PC12 cells from corticosterone-induced lesion at drug concentration of 1.25 μM and the data were shown in Table 1.

Table 1
The protection rates of the final compounds on corticosterone-injured PC12 cells

Compd	*C*Log*P*	Log P	tPSA	PR[a] (%)
4a	4.1	1.53	46.6	34.2 ± 2.1
4b	2.6	1.87	46.6	−16.8 ± 0.9
4c	4.2	2.78	46.4	−50.3 ± 2.0
4d	4.6	1.99	46.4	−12.8 ± 0.4
4e	5.6	2.57	46.4	−10.5 ± 1.1
5a	2.2	1.35	75.6	11.0 ± 8.7
5b	2.4	1.54	75.6	23.2 ± 0.3
5c	3.0	2.01	75.6	2.8 ± 0.3
5d	4.2	1.18	75.6	−8.4 ± 1.1
5e	3.3	1.75	75.6	6.3 ± 1.8
5f	4.1	−1.17	75.6	−17.5 ± 1.4
6a	3.8	2.81	41.6	−11.5 ± 1.0
6b	3.3	−0.52	41.6	9.8 ± 1.5
6c	2.7	1.56	41.6	15.7 ± 1.3
6d	2.2	1.12	70.6	−19.5 ± 1.5
6e	3.2	1.88	44.8	13.6 ± 0.8
6f	3.9	1.35	44.8	−7.7 ± 0.7
6g	4.8	1.06	71.1	6.3 ± 4.2
6h	5.2	2.05	41.6	2.5 ± 0.9
Agomelatine	2.1	1.62	38.3	26.2 ± 2.4
Fluoxetine	4.6	2.55	21.3	19.7 ± 1.5

[a] PR represents the protection rate of the tested compound at the concentration of 1.25 μM, which is calculated from six independent experiments at 24 h after treatment. PR = $\frac{\bar{A}_d - \bar{A}_c}{\bar{A}_c} \times 100\% \pm$ SD where, $\bar{A}_c$ represents the mean absorbance value of six independant experiments of control group merely treated with corticosterone, $\bar{A}_d$ means the mean absorbance value of six independant experiments of test group treated with corticosterone and test drug and SD means the standard deviation.

Imide analogues **4b–e** did not exhibit any protection effects on PC12 cells, sharply opposite to compound **4a**,[22] which possesses an excellent protection rate of 34.2%, better than Agomelatine (PR 2.0%) and Fluoxetine (PR 19.7%). The six-membered ring imide compound **4c** seemed to be extremely unsuitable for the growth of PC12 cells with its highly minus protection rate (−50.3 ± 2.0%). Such minus value of protection rate possibly meant to the toxicity on PC12 cells of the test compound. Among acylamino carboxylic acid analogues **5a–f**, compounds **5a** and **5b** showed moderate activity, with protection rates of 11.0% and 23.2%, respectively. However, introduction of substituents (methyl- or chloro-) to the alkene carbon atom of the sidechain in compound **5b** led to a nearly completely loss of protective effects with a protection rate of 2.8 ± 0.3% for compound **5c** (with two methyl groups introduced), or to a negative effects on PC12 cells growth with a minus protection rates of −8.4 ± 1.1% for compound **5d** (with two chloro-atoms introduced).As for asymmetrical urea analogues **6a–h**, compound **6c** and **6e** displayed noticeable protection effects with PR values of 15.7% and 13.6%, respectively. Such results do not indicate a conclusive structure–activity relationship.

It is known that fundamental physiochemical features of central nervous system (CNS) drugs are related to their ability to penetrate the blood-brain barrier (BBB) affinity.[23] From a medicinal chemical perspective, the ability to design drugs capable of penetrating the BBB and exhibiting the desired biological response is a formidable challenge. Thus early assessment of the physiochemical properties of potential CNS drugs for their ability to cross the BBB is extremely important. Lipophilicity (*C*log*P*) was the first of the descriptors to be identified as important for CNS penetration and the mean value of *C*Log*P* for the marketed CNS drugs is 3.43 and the range is 0.16–6.59.[24] Molecular topological polar surface area (tPSA) is another key descriptor that was shown to correlate well with passive

Table 2
Cytotoxicity on human normal liver L02 cells and human embryonic kidney 293 cells

Compd	IR[a] (%)		Compd	IR[a] (%)	
	L02	293		L02	293
4a	13.8 ± 1.2	9.6 ± 1.9	**5f**	4.1 ± 2.4	13.4 ± 2.2
4b	8.5 ± 0.9	8.1 ± 2.5	**6a**	22.6 ± 1.8	16.6 ± 2.2
4c	10.4 ± 0.9	41.9 ± 3.1	**6b**	19.3 ± 1.5	28.4 ± 4.0
4d	3.4 ± 4.8	17.2 ± 6.1	**6c**	18.4 ± 1.9	28.4 ± 6.3
4e	9.1 ± 0.8	11.5 ± 2.4	**6d**	5.5 ± 1.2	7.1 ± 0.6
5a	3.1 ± 0.8	13.9 ± 3.1	**6e**	20.1 ± 0.6	46.6 ± 4.2
5b	3.4 ± 1.4	33.0 ± 4.2	**6f**	3.1 ± 0.6	3.9 ± 0.7
5c	5.8 ± 2.0	13.2 ± 2.6	**6g**	3.0 ± 0.1	13.3 ± 3.5
5d	3.8 ± 1.6	18.6 ± 0.7	**6h**	8.6 ± 2.7	16.5 ± 4.4
5e	5.7 ± 0.8	34.1 ± 2.1			
Agomelatine	20.2 ± 7.6	26.6 ± 1.6	Fluoxetine	28.5 ± 6.9	55.8 ± 2.9

[a] IR is the mean inhibitory rate calculated from three independent experiments measured at 24 h after treatment with the test compound at the concentration of 80 μM. The viability of the untreated cells was regarded as 100%. Data are expressed as the mean ± SD.

molecular transport through membranes and therefore, allows prediction of transport properties of drugs (Table 1).[25] The mean value of tPSA for the marketed CNS drugs is 40.5 and the range is 4.63–108.[24] Therefore we calculated out the *C*log*P* and tPSA values of the final compounds (Table 1) by the trial version of ChemBioOffice® Ultra 13.0. From Table 1, we can see that all the *C*log*P* and tPSA values of the synthesized analogues fell into the range of the marketed CNS drugs. It is noteworthy to point out that among the compounds with noticeable protective effects on corticosterone-induced PC12 cells, compound **4a** possesses similar log*P* and *C*log*P* values to Fluoxetine, compounds **5a**–**b** have similar log*P* and *C*log*P* values to Agomelatine and compounds **6c**, **6e** exhibit similar log*P*, *C*log*P* and tPSA values to Agomelatine.

The toxicity profile concerning of liver and kidney is usually considered during the process of drug research and development. Herein, the inhibitory effects of the synthesized compounds were evaluated in vitro on human normal liver L02 cells and human embryonic kidney 293 cells. As shown in Table 2, all the tested drugs showed low or no inhibitory effects on the tested human cells at drug concentration of 80 μM. Preferably, compounds **4a** and **5b**, whose protective rates on corticosterone-induced PC12 cells are on the top 2 list of the tested analogues, also showed quite low inhibition on these two normal cells. For example, the cytotoxicity of compound **4a** (IR: 3.8 ± 1.2%) against normal L02 cells was superior to Agomelatine (IR: 20.2 ± 7.6%) and Fluoxetine (IR: 28.5 ± 6.9%), suggested that compound **4a** exhibit less liver toxicity than Agomelatine and Fluoxetine. The kidney cytotoxicity of compound **4a** (IR: 9.6 ± 1.9%) on 293 cells was also lower than Agomelatine (IR: 26.6 ± 1.6) and Fluoxetine (IR: 55.8 ± 2.9), indicated that compound **4a** possess a much better safety profile than the marketed drugs Agomelatine and Fluoxetine.

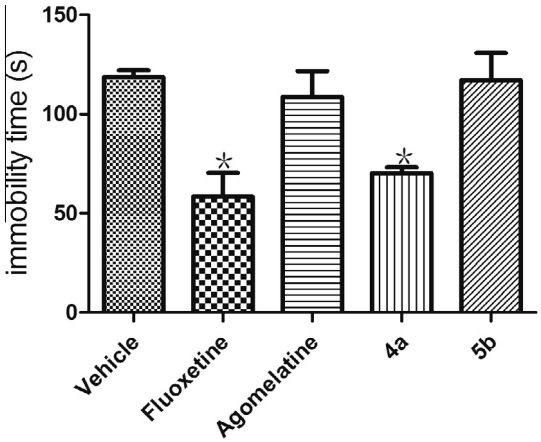


Figure 2. Effect of compounds administrated intraperitoneally (ip) on the immobility time in the forced swim test in C57 mice. Mice were treated on days 2–15 with compound (32 mg/kg/day). Data represent the mean ± SD of 10 mice per group. *$P < 0.01$ versus vehicle.

Based on above *in silico* and *in vitro* data, compounds **4a** and **5b** were selected to further conduct forced swim test and the results were shown in Figure 2. After taken **4a** or **5b** (32 mg/kg/day, suspended in 30% β-Cyclodextrin solution) on days 2–15 by intraperitoneal injection, the immobility time of C57 mice were recorded and analyzed using the Xeye Animal behavior analysis system. Compound **4a** demonstrated more promising to decrease the immobility time of C57 mice than Agomelatine. However, **5b** did not display remarkable in vivo activity, which can reasonably be owed to its poor blood–brain barrier-permeating ability (tPSA: 75.6).

Based on our present study, we hypothesis that the possible mechanism is that target compounds protected PC-12 from lesion and they were antagonist of Glucocorticoid receptor. Certain mechanism still needs further research.

In conclusion, nineteen analogues of Agomelatine were readily synthesized by modification of the amide side-chain and assessed for their potential antidepression activities *in vitro* and *in vivo*. Meanwhile their cytotoxicities on human normal liver L02 cells and human embryonic kidney 293 cells were also tested. *C*log*P*, tPSA and logP were used to predict their ability to penetrate the blood-brain barrier. Based on the *in silico* and *in vitro* results, we chose compounds **4a** and **5b** to further conduct in vivo evaluation. Forced swim test showed that only compound **4a** significantly reduced the immobility time of C57 mice. Our results provide a promising lead (compound **4a**) for subsequent optimization to achieve better efficacy, better pharmacokinetics properties and less adverse effects such as liver toxicity. Meanwhile, subsequent modification of compound **5b** by preparation of its ester or carbamate prodrug may cope with the BBB issue. As for the acting mode of our compounds, we can logically deduce that the antidepressant-like effects may be owed to their antagonism of glucocorticoid receptor, quite different from the mechanism of Agomelatine and Fluoxetine, since corticosterone-induced PC-12 lesion can be significantly attenuated by the treatment of compound **4a** and **5b** in vitro. However, their exact molecular binding mode remained to elucidate in the coming study which can further help us to improve our next generation of compounds.

Acknowledgments

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Supplementary data

Supplementary data (experimental detail and spectra data) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.02.065.

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22. *Spectra of compound* ***4a***: ^{1}H NMR ($CDCl_3$): δ 7.75 (1H, d, J = 7.2 Hz, Ar-H), 7.64 (1H, d, J = 8.0 Hz, Ar-H), 7.57 (1H, d, J = 8.0 Hz, Ar-H), 7.39 (1H, d, J = 6.8 Hz, Ar-H), 7.25 (1H, d, J = 14.4 Hz, Ar-H), 7.15 (1H, dd, J_1 = 9.2 Hz, J_2 = 2.4 Hz, Ar-H), 4.07 (3H, s, –OMe), 3.88 (2H, t, J = 8.4 Hz, $–CH_2–N–$), 3.28 (2H, t, J = 8.4 Hz, $–CH_2–$), 1.98 (6H, s, $–CH_3$, $–CH_3$); ^{13}C NMR (DMSO-d_6): δ 171.62 (–CO–, –CO–), 157.55 ($=C(CH_3)–$, $=C(CH_3)–$), 136.75 (-Ar), 132.87 (-Ar), 132.60 (-Ar), 130.22 (-Ar), 128.77 (-Ar), 127.28 (-Ar), 126.93 (-Ar), 123.15 (-Ar), 118.10 (-Ar), 102.13 (-Ar), 55.16 ($–OCH_3$), 37.76 ($–CH_2–N–$), 31.79 ($–CH_2–$), 8.41 ($–CH_3$, $–CH_3$); MS (TOF) m/z calcd for $C_{19}H_{19}NO_3$ [M+Na$^+$] 332.1263, found: 332.1264.
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