HETEROCYCLES, Vol. 102, No. 1, 2021, pp. 122 - 128. © 2021 The Japan Institute of Heterocyclic Chemistry
Received, 7th November, 2020, Accepted, 30th November, 2020, Published online, 7th December, 2020
DOI: 10.3987/COM-20-14374

IMPROVED SYNTHESIS OF A SMAD3 PHOSPHORYLATION INHIBITOR LINGZHIFURAN A VIA CONDENSATION REACTION

Weiming Chen,[a,b,1] Xiaoling Xu,[a,1] Mingfeng Sun,[c] Xuelong Huang,[a] Weiping Ma,[d,*] and Feng Jiang[a,b,*]

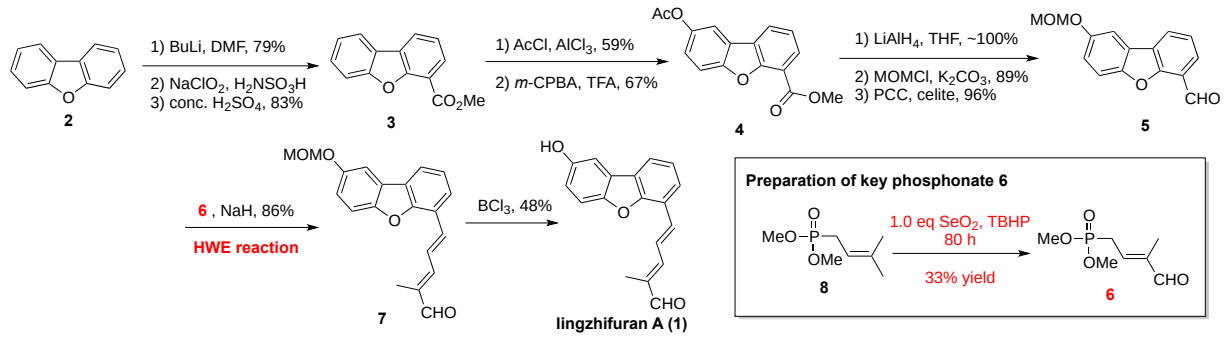
[a]School of Pharmaceutical Sciences, Gannan Medical University, Ganzhou 341000, Jiangxi, China; E-mail: jiangfenghz@163.com
[b]Key Laboratory of Prevention and Treatment of Cardiovascular and Cerebrovascular Disease, Ministry of Education, Gannan Medical University, Ganzhou, 341000, Jiangxi, China
[c]Iyitech Shanghai Co., Ltd., Shanghai 201108, China
[d]School of Stomatology and Medicine, Foshan University, Foshan 528000, Guangdong, China; E-mail: mawp1034@126.com
[1]These authors contributed equally to this work.

Abstract – A facile and high-efficient synthesis of lingzhifuran A, a Smad3 phosphorylation inhibitor isolated from *Ganoderma lucidum*, was developed from commercially available dibenzo[*b*,*d*]furan. The crucial step of this strategy was achieved via condensation reaction using key intermediate 8-hydroxydibenzo[*b*,*d*]furan-4-carbaldehyde and commercially available (*E*)-2-methylbut-2-enal. By this strategy, lingzhifuran A was obtained in 5 steps with up to 57.6% yield.

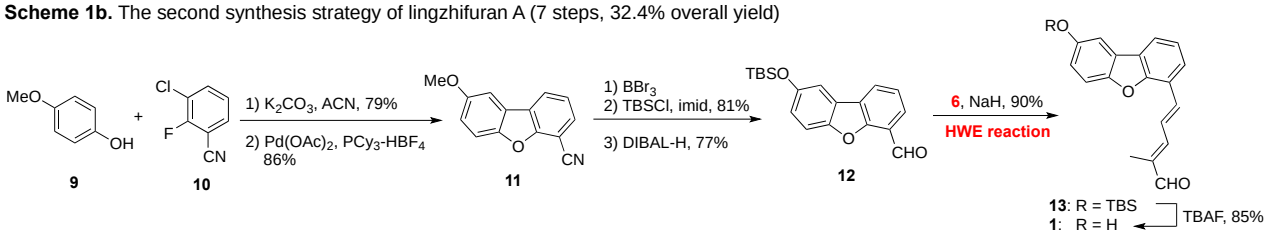
Natural products and traditional medicines are of great importance for drug research.[1] From *Ganoderma lucidum*,[2] a Chinese herb medicine, many phenolic meroterpenoids with biological activities were isolated in recent decades,[3] such as lingzhifuran A (**1**), lingzhiol, lingzhilactone B, lingzhilactone D, sinensilactam A, applanatumin A, applanatumol A, and applanatumol B. Among them, it was found that lingzhifuran A could selectively inhibit TGF-β1-induced Smad3 phosphorylation in rat renal tubular epithelial cells from *in vitro* and *in vivo* experiments, which represented novel scaffolds of selective Smad3 phosphorylation inhibitors.[3a,4]

Considering that lingzhifuran A was trace in *Ganoderma lucidum*, two synthesis strategies were reported by Ding et al.[3a,5] In the first synthesis strategy, starting from dibenzo[*b*,*d*]furan **2**, lingzhifuran A was obtained in 10 steps with 9.1% overall yield (Scheme 1a). For the key step from intermediate **5** to polyunsaturated aldehyde **7**, Horner-Wadsworth-Emmons reaction (HWE reaction) was performed using (*E*)-dimethyl (3-methyl-4-oxobut-2-en-1-yl)phosphonate **6** with 86% yield,[6] but phosphonate **6** was difficult to be prepared from dimethyl (3-methylbut-2-en-1-yl)phosphonate **8** under SeO_2/TBHP oxidation with only 33% yield even up to 80 h.[5,7] The second synthesis strategy was achieved by building up the dibenzo[*b*,*d*]furan core from **9** and **10**, and using HWE reaction also, as shown in Scheme 1b, which was in 7 steps with 32.4% overall yield.[3a] Compared with the first synthesis route, lingzhifuran A was obtained with shorter steps and higher yield, but several harsh conditions were involved, such as Pd-coupling reaction, demethylation and DIBAL-H reduction, which were seriously restricted the scale-up for further research. For further research and drug discovery, a suitable synthetic approach for lingzhifuran A which possess the advantages, such as cost-effective, practical and scalable, is highly desired.

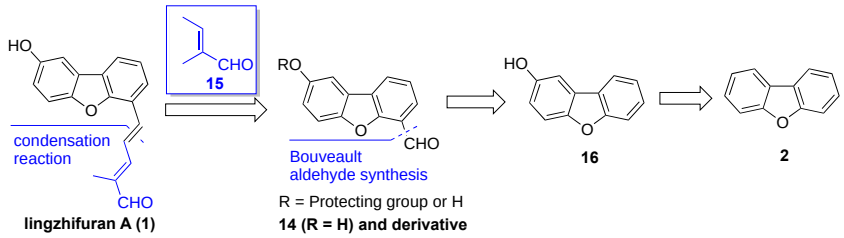
Scheme 1a. The first synthesis strategy of lingzhifuran A (10 steps, 9.1% overall yield)



Scheme 1b. The second synthesis strategy of lingzhifuran A (7 steps, 32.4% overall yield)



Scheme 1c. Retrosynthetic analysis of this work via **condensation reaction**, avoiding HWE reaction

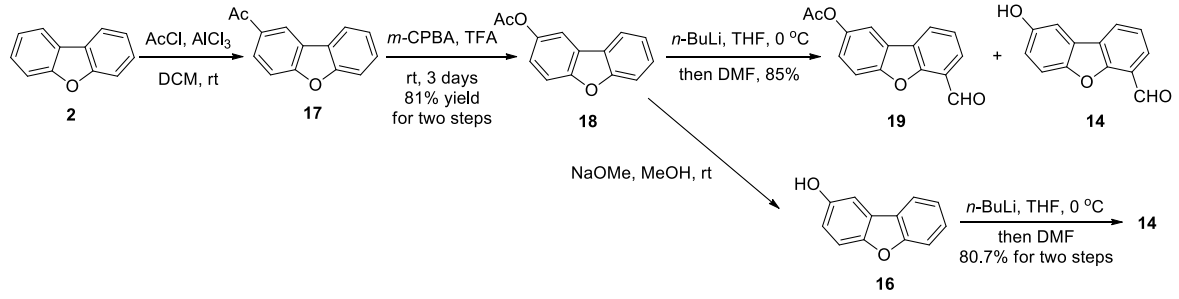


Scheme 1. Reported synthesis strategies and retrosynthetic analysis of this work

Herein we report more efficient synthesis strategy for lingzhifuran A (**1**) in fewer steps via condensation reaction, avoiding HWE reaction. As shown in Scheme 1c, based on the retrosynthetic analysis, lingzhifuran A was able to be obtained via condensation reaction of key intermediate **14** and commercially available (*E*)-2-methylbut-2-enal **15**,[8] which was obviously different from Ding's strategy (HWE reaction). The intermediate **14** could be prepared from 2-hydroxydibenzo[*b*,*d*]furan **16** via Bouveaut aldehyde synthesis reaction, which was obtained from starting material dibenzo[*b*,*d*]furan **2**.

At the beginning of this work (Scheme 2), intermediate **17** was prepared from dibenzo[*b*,*d*]furan **2** by Friedel-Crafts acylation.[9] After extraction, the solution of intermediate **17** in dichloromethane was used directly for Baeyer-Villiger oxidation.[10] The feasible procedure gave 81% yield for the two steps.

In the following trials, when intermediate **18** was treated with *n*-BuLi/DMF,[11] acetylated aldehyde **19** was produced along with deacetylated aldehyde **14**. Optimization of the aldehyde formation conditions also gave a mixture. Therefore, hydrolysis of acetylated **18**, following treatment with *n*-BuLi/DMF gave aldehyde **14** in a high yield.[12] During the experiments, intermediate **16** was not very stable under common storage condition (even below 0 °C) and used directly for the following aldehyde formation reaction.

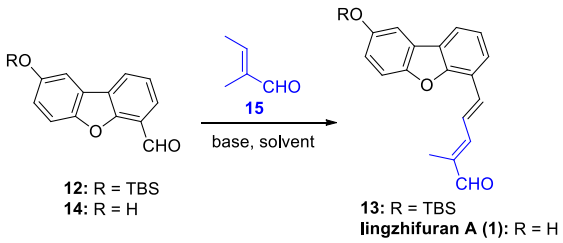


Scheme 2. Synthesis of aldehyde **14** from dibenzo[*b*,*d*]furan **2**

Compared with low yield and tough reaction conditions of phosphonate **6** preparation for HWE reaction, commercially available (*E*)-2-methylbut-2-enal **15** was selected to prepare lingzhifuran A via condensation reaction under basic condition.[8] At the beginning, aldehyde **12** (prepared from **14** using TBSCl) used for HWE reaction was tried to explore condensation reaction using NaH as base. The result indicated that polyunsaturated aldehyde **13** was obtained with 90% conversion rate and 78% isolated yield even after 10 h (Table 1, entry 1). Interestingly, when polar solvent DMF was employed instead of THF, lingzhifuran A was obtained with 68% yield along with 22% of aldehyde **13** (entry 2). The mixture was observed when aqueous NaOH solution was tried to use for the condensation reaction (entry 3).

The results of entries 2 and 3 suggest that de-TBS aldehyde **14** may be involved in the condensation reaction. Fortunately, treatment of aldehyde **14** and enal **15** with aqueous NaOH/THF provided lingzhifuran A in 100% conversion rate and 85% isolated yield (entry 4). As shown in entry 5, instead of THF by EtOH, up to 90% yield of final product was achieved.

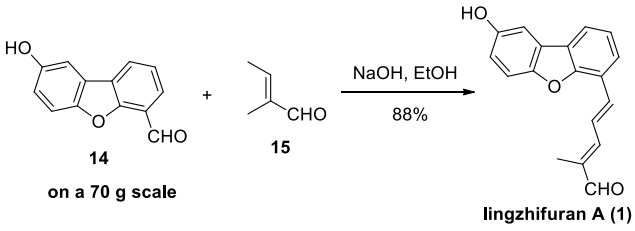
Table 1. The trials of condensation reaction using (*E*)-2-methylbut-2-enal **15**



Entry No.	Substrate	Conditions [a]	Conv. Rate [b]	Product(yield) [c]
1 [d]	**12**	NaH/THF	90%	**13** (78%)
2	**12**	NaH/DMF	>99%	**13** (22%) +**1** (68%)
3	**12**	aq. NaOH/THF	>99%	**13** (75%) + **1** (17%)
4	**14**	aq. NaOH/THF	>99%	**1** (85%)
5	**14**	aq. NaOH/EtOH	>99%	**1** (90%)

Notes: [a] Unless otherwise mentioned, the reaction was performed under the following conditions. NaH (2.0 equiv.) or the solution of NaOH in H_2O (4.0 equiv.) was added into the mixture of the substrate (1.0 g, 4.7 mmol, 1.0 equiv.) in solvent (5.0 mL). (*E*)-2-Methylbut-2-enal **15** (7.1 mmol, 1.5 equiv.) was added dropwise and stirred at 0 °C for 3–5 h. [b] The conversion rate was obtained by HPLC. [c] The isolated yield was calculated after column purification. [d] The reaction time was 10 h.

According to the optimal conditions, the condensation reaction was performed using 1.2 equiv. of (*E*)-2-methylbut-2-enal **15** on a 70 g scale of de-TBS aldehyde **14**. After common workup and simple reslurry with MeOH, lingzhifuran A was obtained as a yellow solid in 88% yield and with 98.0% HPLC purity (Scheme 3).



Scheme 3. Improved synthesis of lingzhifuran A via condensation reaction using **14** and **15**

In summary, starting from commercially available dibenzo[*b*,*d*]furan **2** and performing condensation reaction of aldehyde **14** with commercially available (*E*)-2-methylbut-2-enal **15**, lingzhifuran A (**1**) was

synthesized in only 5 steps with up to 57.6% yield. The improved synthetic method is facile and high-efficient, which is suitable for further research and drug discovery.

EXPERIMENTAL

All commercially available materials and solvents were used directly without further purification unless otherwise noted. ^{1}H NMR and ^{13}C NMR data were recorded with a Bruker spectrometer (400 MHz) using TMS as internal standard and reported relative to residual solvent signals as follows: chemical shift (δ ppm), multiplicity, coupling constant (Hz), and integration. The multiplicities are denoted as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. The ESI mass spectra were determined on a THERMO LTQ.

Preparation of dibenzo[*b,d*]furan-2-yl acetate 18

The solution of 95 g $AlCl_3$ (0.71 mol, 1.2 equiv.) and 55.8 g acetyl chloride (0.71 mol, 1.2 equiv.) in 1000 mL dichloromethane (DCM) was added dropwise into a solution of 100 g dibenzo[*b,d*]furan **2** (0.59 mol, 1.0 equiv.) in 1000 mL DCM. The mixture became brown-red, and was stirred at ambient temperature. After monitored by TLC, the mixture was added into 2000 mL cold hydrochloric acid (0.5 mol/L) under 10 ^{o}C. The resulting yellow organic layer was collected, washed with water and brine, and dried over anhydrous Na_2SO_4. After filtration, the filtrate was used directly for next step.

Into the above solution of **17**, 220 g trifluoroacetic acid (1.93 mol, 3.3 equiv.) was added dropwise under 0 ^{o}C, then 177 g *m*-chloroperbenzoic acid (1.02 mol, 1.7 equiv.) was added. The mixture was stirred at ambient temperature for 3 days till TLC indicated the reaction was finished. Then 450 mL saturated aq. $NaHSO_3$ solution was added dropwise to quench the oxidability. The resulting solid was filtered off and the filtrate was separated to collect the organic phase. After washed with water and dried over anhydrous Na_2SO_4, the filtrate was obtained after filtration and concentrated to give crude product, which was purified by column chromatography to give 108.9 g intermediate **18** (81% yield for two steps). ESI-MS: *m/z* = 227.3 [M+H]. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.90 (d, *J* = 7.3 Hz, 1H), 7.68 (d, *J* = 2.4 Hz, 1H), 7.56 (dd, *J* = 8.3, 7.0 Hz, 2H), 7.51 – 7.43 (m, 1H), 7.34 (t, *J* = 7.5 Hz, 1H), 7.16 (dd, *J* = 8.8, 2.4 Hz, 1H), 2.36 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 169.99, 156.99, 153.66, 146.17, 127.66, 124.99, 123.99, 122.83, 120.87, 120.65, 113.60, 112.12, 111.85, 21.16.

Preparation of 8-hydroxydibenzo[*b,d*]furan-4-carbaldehyde 14

Under nitrogen atmosphere, 100 g intermediate **18** (0.44 mol, 1.0 equiv.) and 35.8 g sodium methoxide (0.66 mol, 1.5 equiv.) were added into 500 mL MeOH. The reaction was stopped after stirred overnight and monitored by TLC. Below 15 ^{o}C, dilute hydrochloric acid (2 mol/L) was added to adjust pH value to

4. The resulting solution was concentrated and extracted with DCM and water. The organic phase was washed with water and brine, and dried over anhydrous Na_2SO_4. After filtration and concentration, crude 77.4 g intermediate **16** was obtained as a yellow solid, which was not stable in air atmosphere and used directly for next step.

In a three-necked flask, 77.4 g intermediate **16** (0.42 mol, 1.0 equiv.) and 75 g activated molecular sieve were mixed with 1000 mL THF. Under nitrogen atmosphere, 530 mL *n*-BuLi in hexane (2.5 mol/L, 1.32 mol, 3.0 equiv.) was added dropwise below -10 °C. After addition, the mixture was warmed to ambient temperature and stirred for 3 h. Then the mixture was cooled to 0 °C and 61.3 g dry DMF (0.84 mol, 2.0 equiv.) was added. The resulting mixture was stirred for 3 h and monitored by TLC. Below 10 °C, hydrochloric acid (2 mol/L) was added to adjust pH = 5. After extracted by EtOAc, the organic phase was treated as common workup. After column chromatography, 75.7 g aldehyde **14** was obtained as a yellow solid (80.7% yield for two steps). ESI-MS: *m/z* = 211.2 [M-H]. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 10.42 (s, 1H), 9.60 (s, 1H), 8.41 (d, *J* = 7.4 Hz, 1H), 7.97 (d, *J* = 7.4 Hz, 1H), 7.62 (d, *J* = 8.8 Hz, 1H), 7.58 – 7.48 (m, 2H), 7.02 (dd, *J* = 8.8, 2.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ= 189.43, 155.59, 154.51, 150.19, 128.86, 127.86, 126.20, 123.51, 123.37, 121.49, 117.05, 112.77, 106.66.

Preparation of lingzhifuran A (1)

Under nitrogen atmosphere, the solution of 52.8 g NaOH (1.32 mol, 4.0 equiv.) in 750 mL purified water was added into the mixture of 70 g aldehyde **14** (0.33 mol, 1.0 equiv.) in 1000 mL EtOH under 0 °C. The mixture was warmed to ambient temperature, and the solution of 33.6 g (*E*)-2-methylbut-2-enal **15** (0.40 mol, 1.2 equiv.) in 200 mL EtOH was added dropwise. The reaction was stopped till HPLC indicated that aldehyde **14** was consumed completely. Below 10 °C, hydrochloric acid (2 mol/L) was added to adjust pH = 4. After extraction with DCM and treatment as common workup, crude product was obtained as a yellow-red solid, which was slurried with 500 mL MeOH to afford 80.7 g lingzhifuran A (**1**) with 88% yield. ESI-MS: *m/z* = 277.0 [M-H]. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 9.52 (d, *J* = 2.4 Hz, 2H), 8.05 (d, *J* = 7.6 Hz, 1H), 7.79 (dd, *J* = 15.6, 9.7 Hz, 2H), 7.59 (d, *J* = 8.8 Hz, 1H), 7.51 – 7.42 (m, 2H), 7.37 (dd, *J* = 17.0, 9.4 Hz, 2H), 6.98 (dd, *J* = 8.8, 2.5 Hz, 1H), 1.93 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 194.90, 153.83, 153.81, 149.22, 149.02, 137.67, 134.76, 126.59, 126.51, 124.62, 123.87, 123.08, 121.92, 120.84, 116.06, 112.18, 106.05, 9.56. The ^{1}H NMR was also measured in acetone-d_6, which met with the publication.[3a] ^{1}H NMR (400 MHz, acetone-d_6) δ 9.59 (s, 1H), 8.47 (s, 1H), 8.03 (dd, *J* = 7.7, 1.2 Hz, 1H), 7.93 (dd, *J* = 15.6, 11.3 Hz, 1H), 7.77 (dt, *J* = 7.6, 1.0 Hz, 1H), 7.58 (d, *J* = 8.8 Hz, 1H), 7.52 (d, *J* = 2.6 Hz, 1H), 7.47 (d, *J* = 15.6 Hz, 1H), 7.39 (t, *J* = 7.7 Hz, 1H), 7.32 (dt, *J* = 11.4, 1.2 Hz, 1H), 7.08 (dd, *J* = 8.8, 2.6 Hz, 1H), 1.99 (d, *J* = 1.1 Hz, 3H).

ACKNOWLEDGEMENTS

This work is supported by the National Natural Science Foundation of China (No. 21961003), Natural Science Foundation of Jiangxi Province (No. 20192BAB205114). The Talent project of Jiangxi Province and Start-up fund of Gannan Medical University are gratefully acknowledged.

REFERENCES

1. (a) J. Zhang, Y. He, X. Jiang, H. Jiang, and J. Shen, *Sci. China. Life Sci*., 2019, **62**, 1332; (b) J. Xie, A.-h. Zhang, H. Sun, G.-l. Yan, and X.-j.Wang, *RSC Adv.*, 2018, **8**, 812.
2. (a) S. El-Mekkawy, M. R. Meselhy, N. Nakamura, Y. Tezuka, M. Hattori, N. Kakiuchi, K. Shimotohno, T. Kawahata, and T. Otake, *Phytochemistry*, 1998, **49**, 1651; (b) N. Yu, Y. Huang, Y. Jiang, L. Zou, X. Liu, S. Liu, F. Chen, J. Luo, and Y. Zhu, *Oxid. Med. Cell. Longev*., 2020, **12**, 1.
3. (a) W.-Y. Ding, J. Ai, X.-L. Wang, F. G. Qiu, Q. Lv, P. Fang, F.-F. Hou, Y.-M. Yan, and Y.-X. Cheng, *RSC Adv.*, 2016, **6**, 77887; (b) Y.-M. Yan, J. Ai, L.-L. Zhou, A. C. K. Chung, R. Li, J. Nie, P. Fang, X.-L. Wang, J. Luo, Q. Hu, F.-F. Hou, and Y.-X. Cheng, *Org. Lett.*, 2013, **15**, 5488; (c) X. Li, X. Liu, X. Jiao, H. Yang, Y. Yao, and P. Xie, *Org. Lett*., 2016, **18**, 1944; (d) D. Chen, X.-M. Li, H.-M. Liu, M.-M. Li, Y.-X. Cheng, and H.-B. Qin, *Tetrahedron Lett.*, 2016, **57**, 2877; (e) W. Shao, J. Huang, K. Guo, J. Gong, and Z. Yang, *Org. Lett.*, 2018, **20**, 1857; (f) Q. Luo, L. Tian, L. Di, Y.-M. Yan, X.-Y. Wei, X.-F. Wang, and Y.-X. Cheng, *Org. Lett.*, 2015, **17**, 1565.
4. Y. Cheng, W. Ding, F. Hou, J. Ai, and X. Wang, CN106279086A, 2016.
5. W.-Y. Ding, Structural Modification and Antirenal Fibrosis Activity of GQ5 & Total Synthesis of Lingzhifuran A and Aspongdopamines, Kunming Institute of Botany, Chinese Academy of Sciences, 2016.
6. A. Mohamed-Hachi, E. About-Jaudet, J.-C. Combret, and N. Collignon, *Synthesis,* 1999, 1188.
7. L.-D. Li, W. Tang, W.-J. Liu, and Z.-B. Zhao, *Chinese J. Org. Chem.*, 2018, **28**, 489.
8. M. J. Riveira and M. P. Mischne, *Chemistry*, 2012, **18**, 2382.
9. T. Keumi, K. Hamanaka, H. Hasegawa, N. Minamide, Y. Inoue, and H. Kitajima, *Chem. Lett.*, 1988, **17**, 1285.
10. S. Kantevari, T. Yempala, P. Yogeeswari, D. Sriram, and B. Sridhar, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 4316.
11. N. Lakshminarayana, Y. R. Prasad, L. Gharat, A. Thomas, S. Narayanan, A. Raghuram, C. V. Srinivasan, and B. Gopalan, *Eur. J. Med. Chem.*, 2010, **45**, 3709.
12. T. Yempala and B. K. Cassels, *Synth. Commun.*, 2016, **46**, 1909.