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Hydroxychalcones as Potential Anti-Angiogenic Agent

Radha Karki, Youra Kang, Chul Hoon Kim,† Kyungsook Kwak, Jung-Ae Kim,* and Eung-Seok Lee*

College of Pharmacy, Yeungnam University, Gyeongsan 712-749, Korea
**E-mail: eslee@yu.ac.kr (E.-S. Lee); jakim@yu.ac.kr (J.-A. Kim)*
†Department of Pharmacology, College of Medicine, Yonsei University, Seoul 120-752, Korea
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Introduction

Chalcones (1,3-diphenyl propenone), the precursors of flavonoids and isoflavonoids, are abundant in edible plants.[1] Various structural modifications can be made in the chalcone template by introducing different substituents in the phenyl rings or replacing the phenyl rings with heterocyclic rings as shown in Figure 1. Depending on the substitution pattern in two aromatic rings a wide range of pharmacological activities have been reported[1,2] such as antibacterial,[3,4] antifungal,[5] antimalarial,[6] antileishmanial,[7] antioxidant,[8,9] anti-inflammatory[10] and anticancer activity.[11-14] One of the mechanisms of the anticancer activity of chalcones is suppression of angiogenesis.[15]

Angiogenesis is a fundamental process by which new blood vessels are formed from existing vessels. It is essential in reproduction, development and wound repair.[16] However many diseases are driven by persistent unregulated angiogenesis such as rheumatoid arthritis, diabetic retinopathy, atherosclerosis, psoriasis, tumor development, and formation of metastases.[17] In 1971, Folkman proposed that tumor growth and metastasis are angiogenesis-dependent. Therefore, blocking angiogenesis could be a strategy to arrest tumor growth. Without blood vessels, tumors cannot grow beyond a critical size or metastasize to another organ.[18,19] In the past decade, anti-angiogenic drug development has attracted more research interest.[20,21] Angiogenesis is regulated by a balance between pro- and anti-angiogenic molecules.[18] One of the most-specific and critical regulators of angiogenesis is vascular endothelial growth factor (VEGF), which regulates endothelial proliferation, invasion into extracellular matrix, tube formation and survival. Therefore, VEGF is a promising target for anti-angiogenic drug development.[17,22]

Many natural and synthetic chalcones with hydroxyl moiety are reported to possess anti-angiogenic activity.[15,23-25] 2',4'-Dihydroxy-6'-methoxy-3',5'-dimethoxychalcone, a component of Chinese herbal medicine, is a promising anti-angiogenic agent targeting VEGFR tyrosine kinase.[23] Similarly 2'-hydroxy-4'-methoxychalcone was reported to display anti-angiogenic activity.[24] In another study, a series of 2',5'-dihydroxychalcones were screened for anti-angiogenic activity where 2-chloro-2',5'-dihydroxychalcone showed the highest cytotoxicity in HUVECs, and also exhibited strong inhibitory effects on the HUVEC tube formation in an *in vitro* model.[25] Various polyphenols found in red wine and green tea were also found to inhibit angiogenesis.[26,27] All these reports on phenolic compounds motivated us to design and synthesize hydroxychalcones for anti-angiogenic activity.

Chalcone and 1,3-diaryl-propenones (Figure 2) were previously synthesized in our research group and reported to have anti-angiogenic activity.[28-30] Among them chalcone (**1**) possessed the highest inhibitory effect on the VEGF-induced

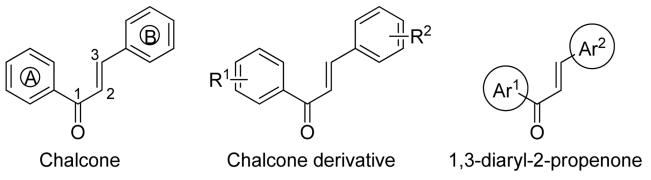


Figure 1. Structures of chalcone, chalcone derivative and 1,3-diaryl-2-propenone.

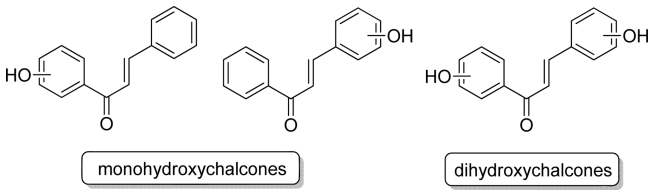


Figure 3. Synthetic strategy for hydroxychalcones.

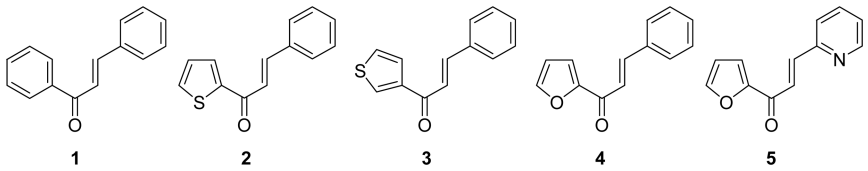


Figure 2. Structures of chalcone and 1,3-diaryl-2-propenones.[28,29]

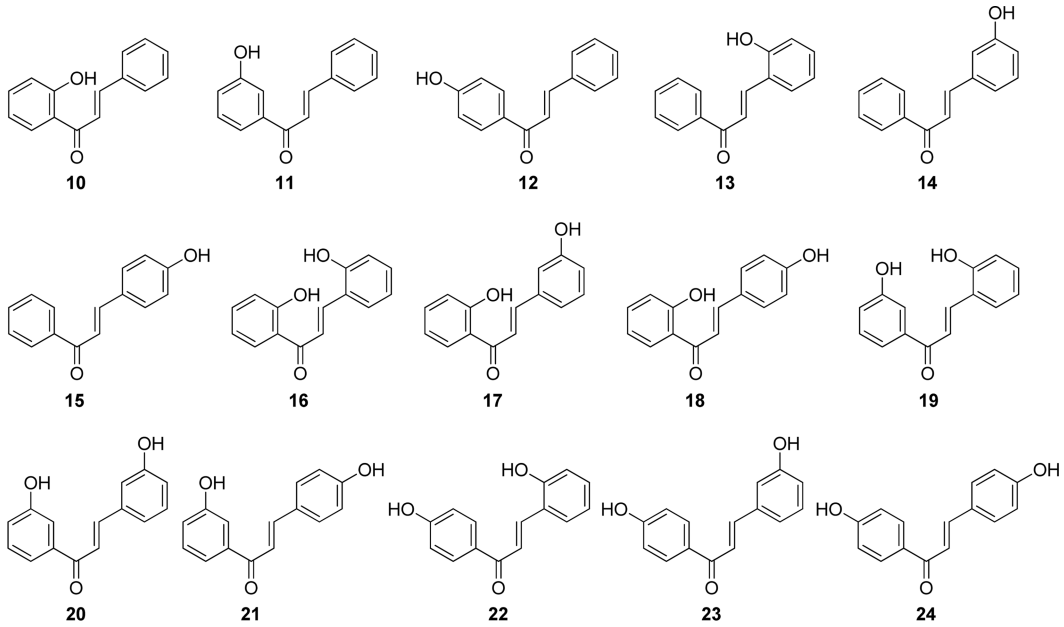


Figure 4. Structures of synthesized hydroxychalcones.[31,32]

angiogenesis.[28] For further optimization of this structure, we have systematically designed and synthesized several mono- and di-hydroxychalcones as shown in Figure 3 and 4 and investigated for anti-angiogenic activity.

Experimental

Material and Methods. Compounds used as starting materials and reagents were obtained from Aldrich Chemical Co., Junsei or other chemical companies, and utilized without further purification. HPLC grade acetonitrile (ACN) and methanol were purchased from Burdick and Jackson, USA. Thin-layer chromatography (TLC) and column chromatography (CC) were performed with Kieselgel 60 F_{254} (Merck) and silica gel (Kieselgel 60, 230-400 mesh, Merck) respectively. Since all the compounds prepared contain aromatic ring, they were visualized and detected on TLC plates with UV light (short wave, long wave or both). NMR spectra were recorded on a Bruker AMX 250 (250 MHz, FT) for ^{1}H NMR and 62.5 MHz for ^{13}C NMR, and chemical shifts were calibrated according to TMS. Chemical shifts (δ) were recorded in ppm and coupling constants (J) in hertz (Hz). Melting points were determined in open capillary tubes on electrothermal 1A 9100 digital melting point apparatus and were uncorrected.

HPLC analyses were performed using two Shimadzu LC-10AT pumps gradient-controlled HPLC system equipped with Shimadzu system controller (SCL-10A VP) and photo diode array detector (SPD-M10A VP) utilizing Shimadzu Class VP program. Sample volume of 10 μL was injected in Waters X-Terra® 5 μM reverse-phase C_{18} column (4.6 250 mm) with a gradient elutions of 50% to 100% of B in A for 10 min followed by 100% to 50% of B in A for 10 min at a flow rate of 1.0 mL/min at 254 nm UV detection, where mobile phase A was double distilled water with 20 mM ammonium formate (AF) and B was 90% ACN in water with 20 mM AF. Purity of compound is described as percent (%) and retention time is given in minutes. ESI MS analysis were performed using an API 4000 LC-MS/MS system (Applied Biosystems, Foster City, CA, USA) equipped with an electrospray ionization interface that was operated in the negative ion mode, $[M–H]^-$.

General Procedure for the Preparation of 8 and 9. Compounds **8** and **9** were synthesized by KOH/NaOH or BF_3-Et_2O catalyzed *Claisen-Schmidt* condensation reaction which were reported earlier by our research group.[31,32]

Method A: To a solution of equimolar amounts of aryl methyl ketone **6** (R^1 = **a-d**) and aryl methyl aldehyde **7** (R^2 = **a-d**) in EtOH was added 50% aqueous solution of KOH (10 equiv) and stirred for 2 to 24 h at 20 °C. The mixture was neutralized with 6 M aqueous HCl solution (pH adjusted to 2), extracted with ethyl acetate, and washed with water and brine. It was further purified by either recrystallization or column chromatography to yield pure solid compounds.

Method B: This method is similar to method A, except the use of 6 M aqueous NaOH solution (5 equiv).

Method C: To a solution of aryl methyl ketone **6** (R^1 = **d**) and aryl methyl aldehyde **7** (R^2 = **d**) in little dioxane was added BF_3-Et_2O (0.5 equiv) gradually at 20 °C and stirred for 2 h. The mixture was then extracted with ethyl acetate and washed with water and brine. It was further purified by column chromatography to yield pure solid compound.

3-(2-Hydroxyphenyl)-1-(3-hydroxyphenyl)propenone (19): The procedure described above (method B) was employed with **6** (R^1 = **c**) and **7** (R^2 = **b**) to yield a green solid (88.2%). mp 173-174 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.47; HPLC: purity 100%, retention time 7.97 min; ESI MS: $[M–H]^-$ = 239.1; ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.00 (br, 2H, 1-phenyl 3'-OH, 3-phenyl 2-OH), 8.02 (d, J = 15.8 Hz, 1H, CO-CH=C**H**), 7.83 (dd, J = 8.1, 1.3 Hz, 1H, 3-

phenyl H-6), 7.79 (d, *J* = 15.8 Hz, 1H, CO-C**H**=CH), 7.55 (d, *J* = 7.7 Hz, 1H, 1-phenyl H-6), 7.40 (t, *J* = 2.2 Hz, 1H, 1-phenyl H-2), 7.35 (t, *J* = 7.9 Hz, 1H, 1-phenyl H-5), 7.26 (td, *J* = 8.5, 1.5 Hz, 1H, 3-phenyl H-4), 7.05 (ddd, *J* = 8.0, 2.4, 0.7 Hz, 1H, 1-phenyl H-4), 6.94 (dd, *J* = 8.2, 0.8 Hz, 1H, 3-phenyl H-3), 6.85 (t, *J* = 7.7 Hz, 1H, 3-phenyl H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 189.66, 157.91, 157.46, 139.68, 139.58, 132.23, 130.08, 129.10, 121.58, 121.38, 120.26, 119.66, 119.56, 116.44, 114.73.

3-(2-Hydroxyphenyl)-1-(4-hydroxyphenyl)propenone (22): The procedure described above (method A) was employed with **6** (R^1 = **d**) and **7** (R^2 = **b**) to yield a red solid (36.4%). mp 197-198 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.33; HPLC: purity 97%, retention time 7.69 min; ESI MS: $[M-H]^-$ = 239.2; ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.32 (br, 2H, 1-phenyl 4'-OH, 3-phenyl 2-OH), 8.02 (d, *J* = 8.7 Hz, 2H, 1-phenyl H-2, H-6), 8.01 (d, *J* = 15.9 Hz, 1H, CO-CH=C**H**), 7.84 (d, *J* = 15.8 Hz, 1H, CO-C**H**=CH), 7.83 (d, *J* = 8.8 Hz, 1H, 3-phenyl H-6), 7.24 (td, *J* = 8.4, 1.4 Hz, 1H, 3-phenyl H-4), 6.92 (d, *J* = 7.5 Hz, 1H, 3-phenyl H-3), 6.89-6.81 (m, 3H, 3-phenyl H-5, 1-phenyl H-3, H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 187.62, 162.16, 157.20, 138.37, 131.85, 131.14, 129.58, 128.70, 121.79, 121.12, 119.57, 116.36, 115.54.

Pharmacology.

Chick Chorioallantoic Membrane (CAM) Model of Angiogenesis: Angiogenesis was examined using previously published method.[33] Briefly, fertilized chicken eggs were incubated at 37 °C with 55% relative humidity. A small hole in the shell concealing the air sac was made using a hypodermic needle. By candling, a second hole was made on the broad side of the egg directly over the avascular portion of the embryonic membrane. A false air sac was created beneath the second hole by applying negative pressure through the first hole, causing the CAM to separate from the shell. An approximately 1.0 cm^2 window was made in the shell over the dropped CAM using a small grinding wheel (Dremel, Racine, WI, USA). VEGF (20 ng/CAM) was used as a standard proangiogenic agent. Sterile disks of No. 1 filter paper (Whatman International) were pretreated with 3 mg/ml of cortisone acetate and air dried under sterile conditions. Each disk was suspended in 10 μL of PBS containing VEGF or the control solvent, and the disks were then placed on growing CAMs. Compounds were added topically to the CAMs 30 minutes later. The drug-treated CAMs were incubated for 3 days.

Results and Discussion

Synthetic Chemistry. Several hydroxychalcones were synthesized by earlier reported methodology.[31,32] Reactions were either base or acid catalyzed, without protection of hydroxyl group. Base catalyzed method employed either 50% aqueous solution of KOH or 6 M NaOH to the solution of equimolar amounts of aryl methyl ketone **6** (R^1 = **a-d**) and aryl methyl aldehyde **7** (R^2 = **a-d**) in ethanol to obtain compounds **8** (R^1, R^2 = **a-d**) in 36-94% yield. Compound **9** (R^1, R^2 = **d**) was obtained in very low yield by this method. Therefore, different methodology was applied using Lewis acid, borontrifluoro etherate (BF_3-Et_2O).[31,32] To a solution of aryl methyl ketone **6** (R^1 = **d**) and aryl methyl aldehyde **7** (R^2 = **d**) in dioxane was added BF_3-Et_2O to obtain compound **9** (R^1, R^2 = **d**) in 74% yield.

Total fifteen compounds (**10-24**) were synthesized as shown in Figure 4. Among them, compounds **10-15** contain single hydroxyl group at *ortho*, *meta* or *para* position on phenyl ring A or B whereas compounds **16-24** possess two hydroxyl groups at *ortho*, *meta* or *para* position on each phenyl ring A and B. Structure-activity relationship (SAR) was determined according to the number and position of hydroxyl group.

Table 1. Inhibitory effects of hydroxychalcones (**10-24**) on the VEGF-induced angiogenesis *in vivo*

Treatment	Compounds (1 μg/CAM)	Vessel Branch points/Main vessel	% Inhibition
PBS		17.9 ± 2.3	
VEGF (20 ng/CAM)		57.7 ± 8.6	
VEGF (20 ng/CAM) +	[a]Chalcone (**1**)		64.3 ± 4.1
	10	43.3 ± 4.0	17.2 ± 12.3
	11	42.7 ± 3.9	18.9 ± 12.2
	12	40.1 ± 5.6	26.9 ± 17.4
	13	51.1 ± 3.7	−7.2 ± 11.6
	14	35.8 ± 3.9	40.5 ± 12.3
	15	27.5 ± 3.6	66.2 ± 11.1
	16	29.1 ± 3.3	71.7 ± 8.2
	17	28.8 ± 3.5	72.5 ± 8.7
	18	31.9 ± 6.7	64.9 ± 1.8
	19	22.2 ± 1.7	89.1 ± 4.3
	20	28.6 ± 5.3	73.1 ± 13.4
	21	25.6 ± 5.4	80.7 ± 13.6
	22	22.8 ± 2.0	87.5 ± 5.1
	23	26.8 ± 4.1	77.5 ± 10.3
	24	27.6 ± 2.6	75.5 ± 6.5

[a]Previously reported result.[28] The data are expressed as the mean ± SEM.

CAM Assay. The synthesized hydroxychalcones (**10-24**) were screened for their inhibitory effect on VEGF-induced angiogenesis *in vivo* using chick chorioallantoic membrane (CAM) and the results are summarized in Table 1. The newly formed blood vessel branch points were significantly increased by VEGF compared with those in the PBS-treated group. The treatment of prepared compounds to CAM strongly inhibited VEGF-induced angiogenesis as indicated by a marked reduction in the number and length of small blood vessels after incubation as shown in Figure 5 and 6.

Among compounds **10-15**, which contain a single hydroxyl group at *ortho*, *meta* or *para* position on phenyl ring, compound **15** displayed the most significant anti-angiogenic activity (66%) *in vivo* that was higher than chalcone (64%) at a dose of 1 μg/CAM. Similarly, compound **14** possessed considerable activity (40%) whereas compounds **10-12** exhibited weak inhibitory activity on VEGF-induced angio-

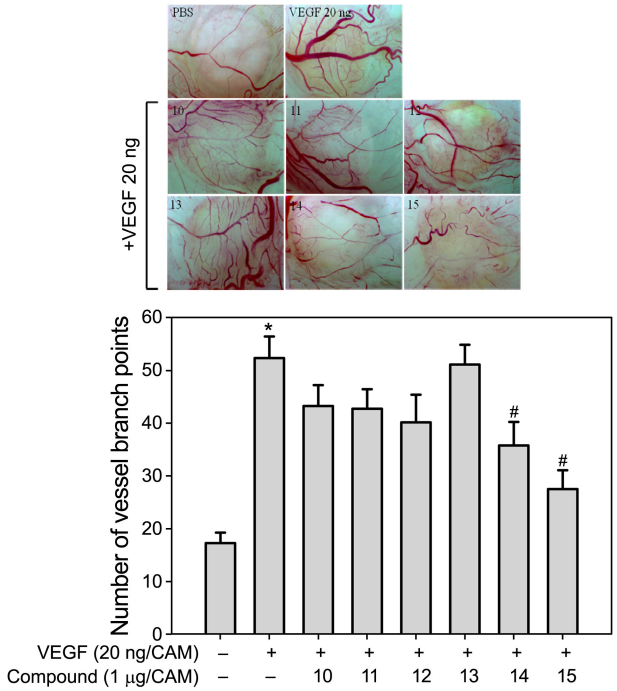


Figure 5. Inhibitory effect of compounds **10-15** on VEGF-induced angiogenesis ($^{*}P < 0.05$ compared to control group, $^{\#}P < 0.05$ compared to VEGF-treated group).

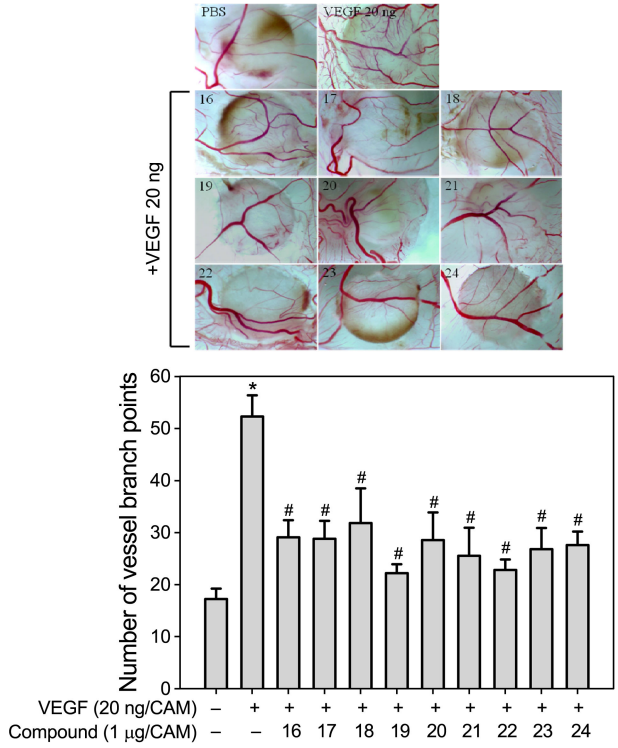
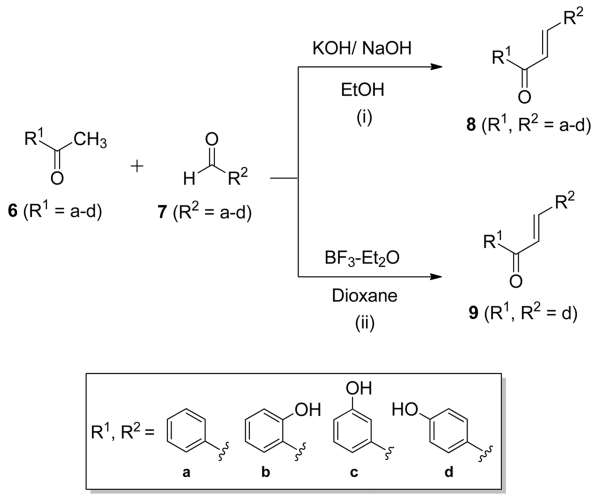


Figure 6. Inhibitory effect of compounds **16-24** on VEGF-induced angiogenesis ($^{*}P < 0.05$ compared to control group, $^{\#}P < 0.05$ compared to VEGF-treated group).

genesis. Compound **13** did not show any inhibitory activity. All the compounds **16-24** which contain two hydroxyl groups at *ortho*, *meta*, or *para* position on each phenyl rings displayed significant inhibitory activity (> 64%) on VEGF-induced angiogenesis which was higher than that of chalcone (**1**). Compound **19** exhibited the highest inhibition (89%) among all the compounds. Similarly, compounds **21** and **22** displayed 80% and 87% inhibition, respectively.



Scheme 1. Synthesis of hydroxychalcones.
Reagents and conditions: (i) KOH (10 equiv)/NaOH (5 equiv), EtOH, 2-24 h, 20 °C, 36-94% yield; (ii) BF_3Et_2O (0.5 equiv), Dioxane, 2 h, 20 °C, 74% yield.

Various natural and synthetic chalcone derivatives have been studied for anti-angiogenic activity. However, this is the first report on the systematic study of hydroxychalcone using CAM model. In the previous study, four phenylpropenone derivatives (**1-4**) were studied and chalcone (**1**) was found to be the most effective. Its action was mediated through the inhibition of receptor tyrosine kinases (RTKs) including VEGF receptor 2.[28] Another propenone compound, FPP-3 (**5**) was reported to exert an anti-angiogenic effect by inhibiting VEGF-induced reactive oxygen species generation and ERK phosphorylation.[29] The inhibitory activity of chalcone (**1**) on angiogenesis *in vivo* was 64% which was much more potent than FPP-3 (**5**) having 29% inhibition at a dose of 1 μg/CAM.

Structure-activity relationship study revealed that dihydroxychalcones exhibited stronger anti-angiogenic activity than monohydroxychalcones and chalcone. Compounds **14** and **15**, which possessed single hydroxyl moiety at *meta* and *para* position, respectively, on phenyl ring B displayed considerable activity but compound **13** with hydroxyl moiety at *ortho* position on phenyl ring B was devoid of activity. It was observed that compounds **19** and **22**, which displayed significant inhibitory activity on VEGF-induced angiogenesis, possessed hydroxyl moiety at *ortho* position on phenyl ring B in common. This suggest that compounds bearing hydroxyl moiety at *ortho* position on phenyl ring B together with *meta* or *para* hydroxyl substitution on phenyl ring A is important for the activity. In addition, all the synthesized compounds possess drug-like properties on the basis of Lipinski's rule of five.[34] Introduction of hydroxyl group decrease the Log P value of compounds that helps to further optimize the basic chalcone template.

Conclusion

Fifteen hydroxychalcones were systematically designed and synthesized using *Claisen-Schmidt* condensation reaction. Compounds were evaluated for their inhibitory effect on VEGF-induced angiogenesis *in vivo* using chick chorioallantoic membrane (CAM). SAR study revealed that dihydroxychalcones were more potent than chalcone and monohydroxychalcones. Further exploration and optimization of chalcone template may produce more potent anti-angiogenic compound.

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References

1. Katsori, A. M.; Litina, D. H. *Expert Opin. Ther. Patents* **2011**, *21*, 1575.
2. Dimmock, J. R.; Elias, D. W.; Beazely, M. A.; Kandepu, N. M. *Curr. Med. Chem.* **1999**, *6*, 1125.
3. Avila, H. P.; Smania, E. F. A.; Monache, F. D.; Junior, A. S. *Bioorg. Med. Chem*. **2008**, *16*, 9790.
4. Usta, A.; Yasar, A.; Yilmaz, N.; Gulec, C.; Yayh, N.; Karaoglu, S. A.; Yayh, N. *Helv. Chim. Acta* **2007**, *90*, 1482.
5. Batovska, D.; Parushev, S.; Slavova, A.; Bankova, V.; Tsvetkova, I.; Ninova, M.; Najdenski, H. *Eur. J. Med. Chem.* **2007**, *42*, 87.
6. Liu, M.; Wilairat, P.; Go, M. L. *J. Med. Chem.* **2001**, *44*, 4443.
7. Zhai, L.; Chen, M.; Blom, J.; Theander, T. G.; Christensen, S. B.; Kharazmi, A. *J. Antimicrob. Chemother.* **1999**, *43*, 793.
8. Dinkova-Kostova, A. T.; Massiah, M. A.; Bozak, R. E.; Hicks, R. J.; Talalay, P. *Proc. Natl. Acad. Sci.* **2001**, *98*, 3404.
9. Calliste, C. A.; Le Bail, J. C.; Trouillas, P.; Pouget, C.; Habrioux, G.; Chulia, A. J.; Duroux, J. L. *Anticancer Res.* **2001**, *21*, 3949.
10. Ballesteros, J. F.; Sanz, M. J.; Ubeda, A.; Miranda, M. A.; Iborra, S.; Paya, M.; Alcaraz, M. J. *J. Med. Chem.* **1995**, *38*, 2794.
11. Srinivasan, B.; Johnson, T. E.; Lad, R.; Xing, C. *J. Med. Chem.* **2009**, *52*, 7228.
12. Cabrera, M.; Simoens, M.; Falchi, G.; Lavaggi, M. L.; Piro, O. E.; Castellano, E. E.; Vidal, A.; Azqueta, A.; Monge A.; Cerain, A. L.; Sagrera, G.; Seoane, G.; Cerecetto, H.; Gonzalez, M. *Bioorg. Med. Chem*. **2007**, *15*, 3356.
13. Rao, Y. K.; Fang, S. H.; Tzeng, Y. M. *Bioorg. Med. Chem*. **2009**, *17*, 7909.
14. Sabzevari, O.; Galati, G.; Moridani, M. Y.; Siraki, A.; O'Brien, P. J. *Chem. Biol. Interact.* **2004**, *148*, 57.
15. Mojzis, J.; Varinska, L.; Mojzisova, G.; Kostova, I.; Mirossay, L. *Pharmacol. Res.* **2008**, *57*, 259.
16. Folkman, J.; Shing, Y. *J. Biol. Chem*. **1992**, *267*, 10931.
17. Pandya, N. M.; Dhalla, N. S.; Santani, D. D. *Vas. Pharmacol.* **2006**, *44*, 265.
18. Carmeliet, P.; Jain, R. K. *Nature* **2000**, *407*, 249.
19. Hanahan, D.; Weinberg, R. A. *Cell* **2000**, *100*, 57.
20. Carmeliet, P. *Nature* **2005**, *438*, 932.
21. Sayed, K. A. E. *Mini-Rev. Med. Chem*. **2005**, *5*, 971.
22. Terman, B. I.; Stoletov, K. V. *J. Biol. Med.* **2001**, *18*, 59.
23. Zhu, X. F.; Xie, B. F.; Zhou, J. M.; Feng, G. K.; Liu, Z. C.; Wei, X. Y.; Zhang, F. X.; Liu, M. F.; Zeng, Y. X. *Mol. Pharmacol.* **2005**, *67*, 1444.
24. Lee, Y. S.; Lim, S. S.; Shin, K. H.; Kim, Y. S.; Ohuchi, K.; Jung, S. H. *Biol. Pharm. Bull.* **2006**, *29*, 1028.
25. Nam, N. H.; Kim, Y.; You, Y. J.; Hong, D. H.; Kim, H. M.; Ahn, B. Z. *Eur. J. Med. Chem.* **2003**, *38*, 179.
26. Oak, M. H.; Bedoui, J. E.; Schini-Kerth, V. B. *J. Nutr. Biochem.* **2005**, *16*, 1.
27. Liu, L.; Lia, C. Q.; Nie, L.; Ordovas, J.; Band, M.; Moser, L.; Meydani, M. *Mol. Nutr. Food Res.* **2008**, *52*, 1182.
28. Lee, J. S.; Kang, Y.; Kim, J. T.; Thapa, D.; Lee, E. S.; Kim, J. A. *Eur. J. Pharmacol.* **2012**, *677*, 22.
29. Park, B. C.; Park, S. Y.; Lee, J. S.; Mousa, S. A.; Kim, J. T.; Kwak, M. K.; Kang, K. W.; Lee, E. S.; Choi, H. G.; Yong, C. S.; Kim, J. A. *Vascul. Pharmacol.* **2009**, *50*, 123.
30. Park, B. C.; Thapa, D.; Lee, Y. S.; Kwak, M. K.; Lee, E. S.; Choi, H. G.; Yong, C. S.; Kim, J. A. *Eur. J. Pharmacol.* **2007**, *567*, 193.
31. Karki, R.; Thapa, P.; Kang, M. J.; Jeong, T. C.; Nam, J. M.; Kim, H. L.; Na, Y.; Cho, W. J.; Kwon, Y.; Lee, E. S. *Bioorg. Med. Chem.* **2010**, *18*, 3066.
32. Karki, R.; Thapa, P.; Yoo, H. Y.; Kadayat, T. M.; Park, P. H.; Na, Y.; Lee, E.; Jeon, K. H.; Cho, W. J.; Choi, H.; Kwon, Y.; Lee, E. S. *Eur. J. Med. Chem.* **2012**, *49*, 219.
33. Cloman, R. W.; Pixley, R. A.; Sainz, I. M.; Song, J. S.; Isordia-Salas, I.; Muhamed, S. N.; Powell, J. A., Jr.; Mousa, S. A. *J. Thromb. Haemost.* **2003**, *1*, 164.
34. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Deliv. Rev.* **1997**, *23*, 3.