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# Polyphosphoric acid-promoted one-pot synthesis and neuroprotective effects of flavanones against NMDA-induced injury in PC12 cells†

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We report herein an efficient polyphosphoric acid (PPA) promoted one-pot protocol for the synthesis of flavanone derivatives from 2-hydroxyacetophenones and benzaldehydes. A variety of flavanones were produced in moderate to excellent yields and evaluated for their neuroprotective effects against *N*-methyl-D-aspartate (NMDA)-induced excitotoxicity in PC12 cells. Derivatives bearing electron-donating groups exhibited better neuroprotective activity. Compound **3m** demonstrated the best protective potency and reversed the intracellular calcium ( $\text{Ca}^{2+}$ ) influx caused by NMDA, suggesting that flavanones protected the PC12 cells against NMDA-induced neurotoxicity *via* inhibition of  $\text{Ca}^{2+}$  overload.

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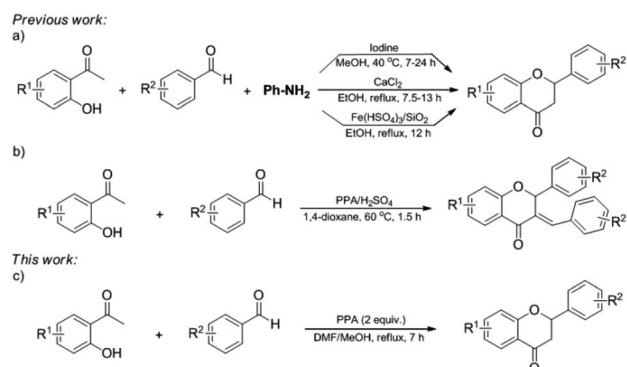
## Introduction

Flavanone derivatives are found in numerous natural and synthetic products.<sup>1</sup> They also are building blocks in the synthesis of biologically active compounds.<sup>2</sup> Most flavanones display varied bioactivities such as anti-cancer,<sup>3</sup> anti-inflammatory,<sup>4</sup> anti-oxidant,<sup>5</sup> and anti-microbial,<sup>6</sup> to name a few. Therefore, the synthesis of flavanones has gained widespread attention. Several methods for construction of flavanones to make use of acid catalysts,<sup>7</sup> bases,<sup>8</sup> oxidants,<sup>9</sup> transition-metal catalysts,<sup>10</sup> and thermal, photochemical, and electrochemical transformations<sup>11</sup> have been established. However, special starting materials such as phenyl alkenyl aryl ethers,<sup>10a</sup> 2-(benzo [*d*]thiazol-2-ylsulfonyl)-1-phenylethanones,<sup>8e</sup> and arynes<sup>12</sup> are required for this approach. Recently, several one-pot procedures for the preparation of flavanones *via* aniline-mediated cyclization of 2-hydroxyacetophenones and benzaldehydes have been reported (Scheme 1a).<sup>13</sup> Despite significant advances, the addition of aniline and longer reaction time are required. Therefore, it is still desirable to develop new efficient catalytic systems.

The *N*-methyl-D-aspartate (NMDA) receptor belongs to ionotropic glutamate receptor that plays a role in brain development, brain function, learning and memory *via*  $\text{Ca}^{2+}$  influx.<sup>14</sup>

The excessive stimulation of the NMDA receptor leading to  $\text{Ca}^{2+}$  overload of neurons resulting neuronal death, which is responsible for a number of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and depression.<sup>15</sup> High concentration of NMDA can overactivation of NMDA receptor resulting the neuronal damage. PC12 cell line is one of the widely used neuronal cell lines in the evaluation of neuroprotective effects of NMDA receptor inhibitors against NMDA-induced injury.

Our group has developed an efficient and practical PPA/ $\text{H}_2\text{SO}_4$  promoted one-pot synthesis of (*E*)-3-benzylidene flavanones (Scheme 1b),<sup>16</sup> which inspired us to explore the one-pot synthesis of flavanones. Although the synthesis of flavanone *via* the cyclization of chalcone using PPA have been reported,<sup>7d</sup> we wish to describe another efficient one-pot procedure promoted by PPA for the synthesis of flavanone derivatives directly from readily available 2-hydroxyacetophenones and



**Scheme 1** (a) One-pot approaches for the synthesis of flavanones. (b) One-pot synthesis of (*E*)-3-benzylidene flavanones. (c) This work.

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benzaldehydes (Scheme 1c). The neuroprotective activity of synthesized flavanones was evaluated using NMDA-induced PC12 cells injury model.

## Results and discussion

### Chemical synthesis

Our initial studies were carried out with the commercially available 2-hydroxyacetophenone **1a** and benzaldehyde **2a** as test substrates. A mixture of **1a** (1 equiv.), **2a** (4 equiv.) and PPA (4 equiv.) in *N,N*-dimethylformamide (DMF) and MeOH refluxed for 5 h under a nitrogen atmosphere to give the condensation product **3a** in 50% yield (see the ESI, Table S1, entry 1†). Control experiment was performed to further improve the yield, the catalysts and additive such as P<sub>2</sub>O<sub>5</sub>, H<sub>3</sub>PO<sub>4</sub> and H<sub>2</sub>SO<sub>4</sub> were evaluated, PPA gave the best results (Table S1†). Then, we explored the effects of PPA amount, temperature and time. Increasing the amount of PPA to 6 equiv. had no obvious effect on the reaction (Table 1, entry 1). Delightfully, we found that decreasing the amount of PPA further increased the yield (Table 1, entry 2). However, further decreasing the amount of PPA resulted in lower yield (Table 1, entry 3). Subsequently, we investigated the effect of reaction temperature and a decreased yield was observed upon decreasing or increasing the temperature to 100 or 200 °C (Table 1, entries 4 and 5). We then investigated the impact of the reaction time. Fortunately, the desirable compound **3a** was obtained in 84% yield when the reaction time increased to 7 h (Table 1, entry 6). However, prolonging the time (9 h) had a slight reduction in yield (Table 1, entry 7). We then evaluated the reaction using microwave irradiation conditions, 25% of **3a** was isolated (Table 1, entry 8). We further evaluated the effects of protic, aprotic, dipolar aprotic and non-polar aprotic solvents. The results showed that DMF and MeOH were the best choice (Table 1, entry 6 vs. Table S2†). Finally, the optimized reaction conditions were

determined as **1a** (1 equiv.), **2a** (4 equiv.) and PPA (2 equiv.) in DMF and MeOH refluxed for 7 h under a nitrogen atmosphere (Table 1, entry 6). With the optimized reaction conditions in hand, a gram scale reaction was carried out and provided the product **3a** in 73% yield (Table 1, entry 9).

Under the optimized conditions, the substrate scope of this cyclization was examined by varying 2-hydroxyacetophenones **1** and benzaldehydes **2** (Table 2). As shown in Table 2, the reaction proceeded well with a variety of substrates to yield desired flavanone derivatives in moderate to excellent yields. The electronic properties of the substituents have an effect on the reaction yield. We found that electron-donating groups (Me, ethyl and OMe) on 2-hydroxyacetophenones **1** and/or benzaldehydes **2** provided higher yields (**3g**, **3h** and **3k–3o**). Hydroxyl-substituted benzaldehydes had limited influence on the reactivity (**3i**). However, electron-withdrawing groups, such as F, Cl, Br and CF<sub>3</sub>, gave lower yields (**3b–3f** and **3j**). It is worthy to note that aliphatic aldehydes, such as cyclohexanecarboxaldehyde and propanal also underwent smooth transformation to afford the corresponding flavanones in moderate yields (**3p** and **3q**).

Table 2 Evaluation of substrate scope<sup>a</sup>

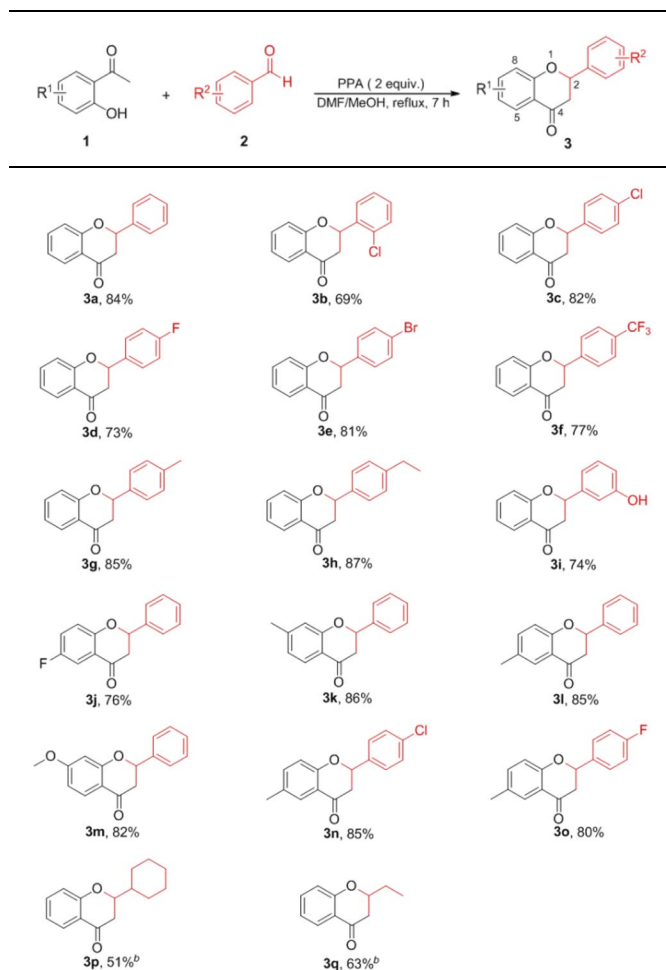


Table 1 PPA amount, temperature and time optimization<sup>a</sup>

Reaction scheme showing the synthesis of flavanone **3a** from 2-hydroxyacetophenone **1a** and benzaldehyde **2a** using PPA in DMF/MeOH.

Entry	PPA (equiv.)	T (°C)	Time (h)	<b>3a</b> <sup>b</sup> (%)
1	6	Reflux	5	48
2	2	Reflux	5	57
3	1	Reflux	5	21
4	2	100	5	33
5 <sup>c</sup>	2	200	5	18
6	2	Reflux	7	84
7	2	Reflux	9	82
8 <sup>d</sup>	2	Reflux	7	25
9 <sup>e</sup>	2	Reflux	7	73

<sup>a</sup> Reaction conditions: **1a** (0.2 mmol), **2a** (0.8 mmol), DMF (6.4 mL), MeOH (1.6 mL). <sup>b</sup> Isolated yield. <sup>c</sup> Reaction carried out in a pressure-resistant reaction bottle. <sup>d</sup> Under microwave irradiation. <sup>e</sup> Reaction conditions: **1a** (8 mmol), **2a** (32 mmol), DMF (16 mL), MeOH (4 mL).

<sup>a</sup> Reaction conditions: **1** (0.2 mmol), **2** (0.8 mmol), PPA (0.4 mmol), DMF (6.4 mL), MeOH (1.6 mL). <sup>b</sup> Reaction time (8 h).



Thus, PPA is an effective catalyst for the one-pot synthesis of flavanones from 2-hydroxyacetophenones and benzaldehydes. PPA not only served as an acid catalyst but also widely used in dehydration condensation and cyclization reactions.<sup>17</sup> As other acids, PPA promotes the enolization of ketone, and, perhaps more importantly, the dehydration and cyclization properties of it are responsible for the efficiency of this one-pot procedure.

The preliminary kinetic experiment of the formation of **3a** was conducted under the standard condition. The yields were determined by <sup>1</sup>H NMR with TTCE as internal standard. As depicted in Fig. 1, the conversion of 2-hydroxyacetophenone **1a** and benzaldehyde **2a** to flavanone **3a** was occurred slowly in the

course of 0.5 h heating and afterwards turned faster. In the late stage, a decreased conversion rate was observed.

A plausible mechanism for the one-pot synthesis of flavanone derivatives is depicted in Scheme 2. Firstly, intermediate **3** is formed from the enolization of 2-hydroxyacetophenone **1a**. Then, the intermediate **3** coupled with benzaldehyde **2a** followed by protonation to produce intermediate **4**, which after subsequent dehydrative cyclisation promoted by PPA gives desired product **3a**.

## Biochemistry

**General information.** Fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), penicillin and streptomycin were obtained from Gibco (Gibco, Paisley, UK). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), NMDA and Fura-2 acetoxyethyl ester (Fluo-2/AM) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). MK-801 was from MCE (Shanghai, China).

**Biological evaluation.** Previous studies shown that flavonoid-rich plant or food extracts have exhibited improvements in cognition and learning,<sup>18</sup> and that flavanone hesperetin has been observed to inhibit oxidative stress-induced neuronal apoptosis.<sup>19</sup> Structurally, flavanones and most glycine antagonists are of a planar structure and flavanone **3a** docked well to the glycine binding site of NMDA receptor (see ESI Fig. S1†). These inspired us to explore the neuroprotective effects of flavanones against NMDA-induced injury in PC12 cells (purchased from Institute of Cell Biology, Chinese Academy of Sciences, Shanghai, China) (Fig. 2).

NMDA injury was performed according to our previous reported protocol<sup>20</sup> and reported in ESI.† The protective effects of

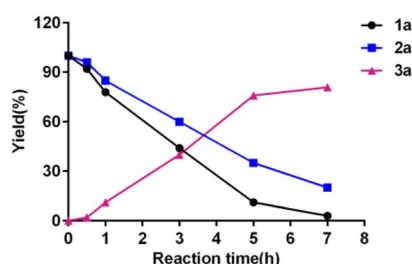
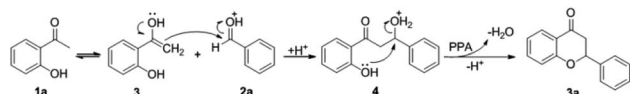


Fig. 1 Kinetic of the formation of flavanone **3a**.



Scheme 2 Plausible mechanism.

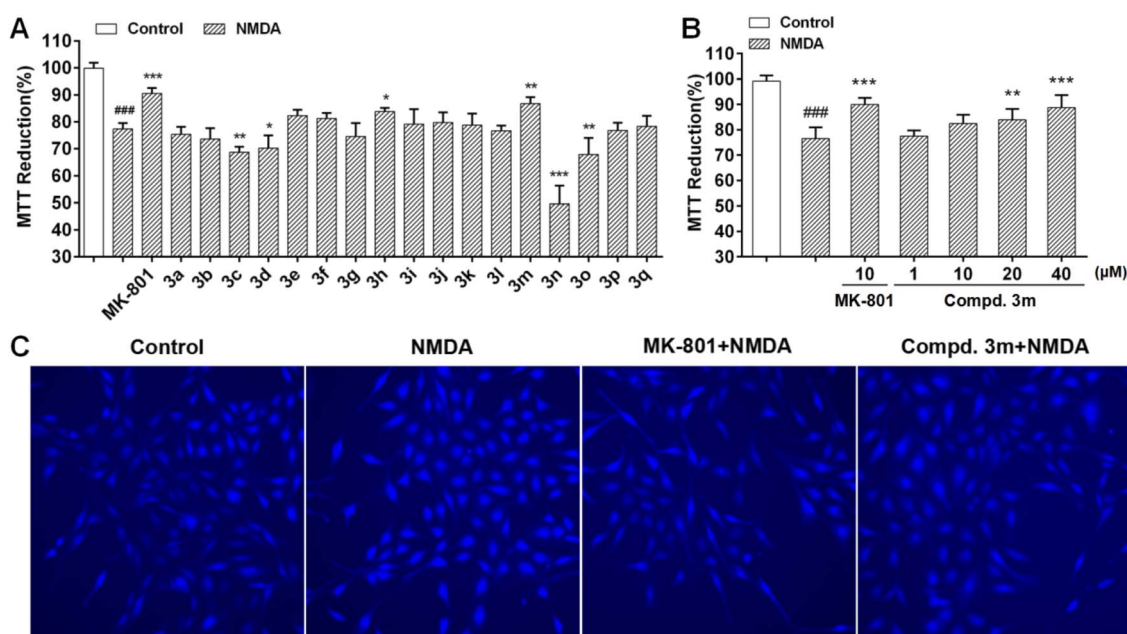


Fig. 2 Protective and Ca<sup>2+</sup> antagonistic effects of flavanones on NMDA-induced injury in PC12 cells. (A) Protective effects of MK-801 and flavanones at 20 μM. (B) Concentration-dependent protective effects of compound **3m**. (C) Blue fluorescence shown the Ca<sup>2+</sup> influx. Cytotoxicity was measured by MTT assay. Data are presented as mean ± SD (*n* = 3). ###*p* < 0.001 as compared to control group, \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001 as compared to NMDA group.





the synthesized flavanones were evaluated *via* the MTT assay. Compounds with electron-donating group (ethyl or OMe) shown a significant increase in the cell viability as compared to NMDA treatment group, and which exhibited better protective activity than that bearing electron-withdrawing group (F, Cl or  $\text{CF}_3$ ) (Fig. 2A, **3h** and **3m** vs. **3b–3f** and **3n, 3o**).

Subsequently, concentration-dependent protective effects of the most active compound **3m** on NMDA-induced damage was carried out (Fig. 2B). At 20  $\mu\text{M}$ , there was a significant increase in the cell viability for compound **3m** compared to NMDA treated group. It was found that pretreatment 40  $\mu\text{M}$  compound **3m** shown the similar neuroprotective potency to the NMDA receptor non-competitive antagonist MK-801 (10  $\mu\text{M}$ ). Then we evaluated the effect of compound **3m** on NMDA-induced  $\text{Ca}^{2+}$  influx in PC12 cells (Fig. 2C). In the present study, the influx of  $\text{Ca}^{2+}$  was inhibited by MK-801 (10  $\mu\text{M}$ ) or compound **3m** (40  $\mu\text{M}$ ), suggesting that the neuroprotective effects of compound **3m** may *via*  $\text{Ca}^{2+}$  antagonism.

### Molecular docking study

Molecular docking study between compound **3m** and glycine binding site of NMDA receptor was conducted. Docking studies of (2*R*)-**3m** and (2*S*)-**3m** were conducted. The results indicated that (2*R*)-**3m** (green) and (2*S*)-**3m** (gray) fitted well in the active pocket of NMDA receptor and exhibited binding energy of  $-7.14$  and  $-6.55$  kcal mol $^{-1}$  respectively (Fig. 3A). In the binding mode, (2*R*)-**3m** formed three hydrogen bonds with the amino acid residues ARG-131, THR-126 and SER-180 (Fig. 3B and D). It was observed that (2*R*)-**3m** being stabilized by Pi-stacking interaction with PHE-92 (Fig. 3B). The orientation and interactions network of (2*S*)-**3m** were little different from (2*R*)-**3m**, but the 2-phenyl part was overlapped (Fig. 3A). (2*S*)-**3m** was able to form the hydrogen bonding interaction with the residues SER-180 and ASP-224 (Fig. 3C and E). Additionally, the 2-phenyl moiety of (2*R*)-**3m** or (2*S*)-**3m** was embedded in the hydrophobic pocket formed by residues TRP-223, PHE-16 and PHE-250 (Fig. 3B and C).

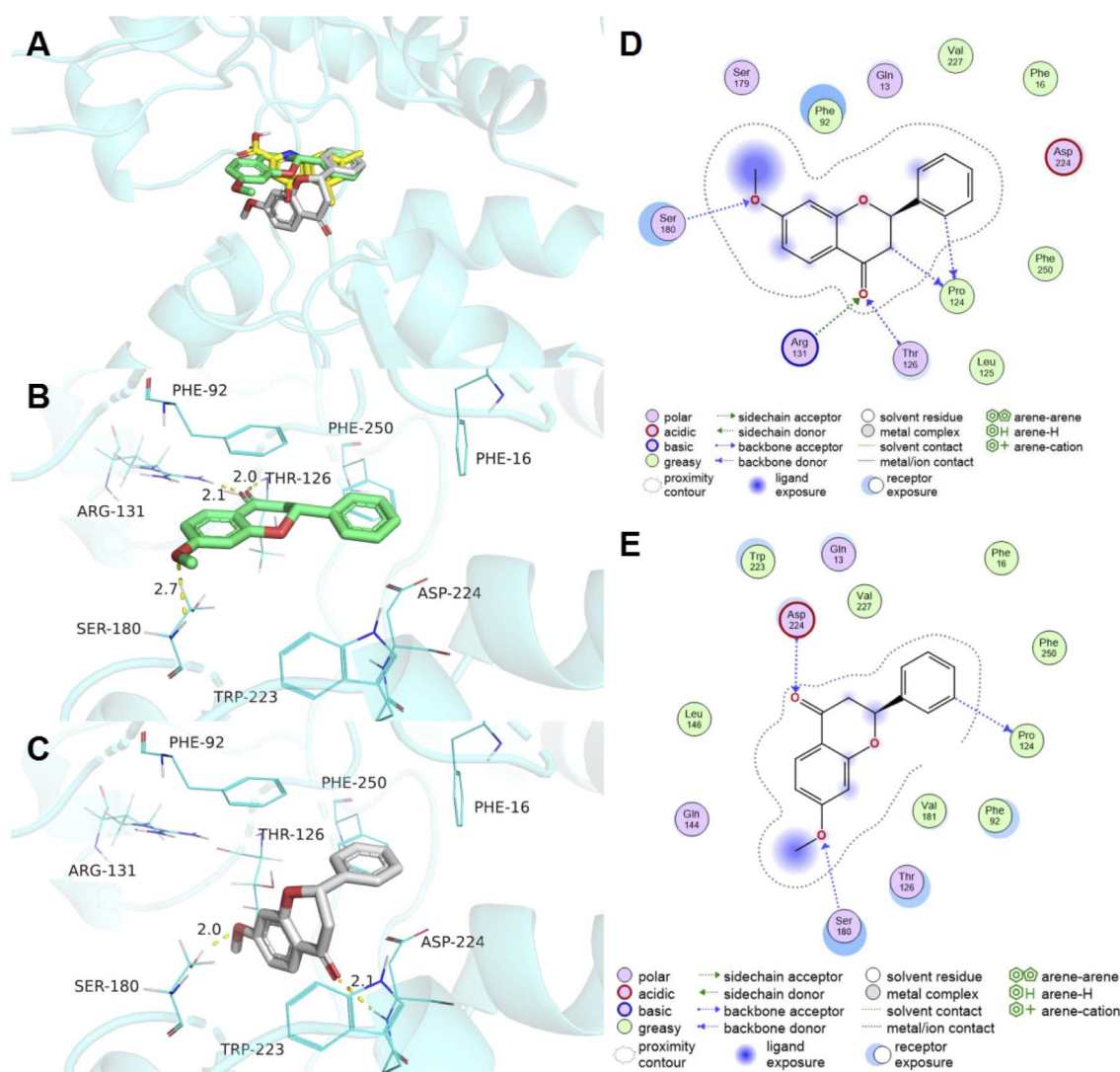


Fig. 3 Docking of compound **3m** with glycine binding site of NMDA receptor (PDB: 4NF4). (A) Alignment of (2*R*)-**3m** (green), (2*S*)-**3m** (gray) and co-crystallized ligand DCKA (yellow) in the active site. The interactions of (2*R*)-**3m** (B) and (2*S*)-**3m** (C) with the active site residues. Hydrogen bond interactions were shown in yellow dotted lines. 2D diagram of (2*R*)-**3m** (D) and (2*S*)-**3m** (E) interactions with the active site residues.



## Conclusions

In conclusion, we have developed an efficient PPA-promoted one-pot approach for the preparation of flavanone derivatives from 2-hydroxyacetophenones and benzaldehydes. In addition, the neuroprotective effect of the synthesized flavanone derivatives against NMDA-induced injury in PC12 cells was evaluated. Derivatives with electron-donating groups shown a significant protective potency. Notably, 7-OMe substituted compound **3m** exhibited good protective effects and could inhibit the excessive intracellular Ca<sup>2+</sup> caused by NMDA.

## Conflicts of interest

There are no conflicts to declare.

## Acknowledgements

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## Notes and references

- See for example: (a) G. U. Siddikov, M. P. Yuldashev, S. F. Aripova, A. D. Vdovin, N. D. Abdullaev and E. K. Botirov, *Chem. Nat. Compd.*, 2008, **44**, 28; (b) Z. Zhou, P. Zhao, W. Huang and G. Yang, *Adv. Synth. Catal.*, 2006, **348**, 63; (c) A. Paredes, M. Alzuru, J. Mendez and M. Rodríguez-Ortega, *Biol. Pharm. Bull.*, 2003, **26**, 108.
- W. D. Z. Li and B. C. Ma, *Org. Lett.*, 2005, **7**, 271.
- (a) A. Maiti, M. Cuendet, V. L. Croy, D. C. Endringer, J. M. Pezzuto and M. Cushman, *J. Med. Chem.*, 2007, **50**, 2799; (b) M. Cabrera, M. Simoens, G. Falchi, M. L. Lavaggi, O. E. Piro, E. E. Castellano and M. Gonzalez, *Bioorg. Med. Chem.*, 2007, **15**, 3356.
- (a) H. K. Hsieh, T. H. Lee, J. P. Wang, J. J. Wang and C. N. Lin, *Pharm. Res.*, 1998, **15**, 39; (b) D. Njamen, J. T. Mbafor, Z. T. Fomum, A. Kamanyi, J. C. Mbanya, M. C. Recio and J. L. Ríos, *Planta Med.*, 2004, **70**, 104.
- H. A. Jung, D. M. Jeong, H. Y. Chung, H. A. Lim, J. Y. Kim, N. Y. Yoon and J. S. Choi, *Biol. Pharm. Bull.*, 2008, **31**, 908.
- F. E. Ward, D. L. Garling, R. T. Buckler, D. M. Lawler and D. P. Cummings, *J. Med. Chem.*, 1981, **24**, 1073.
- (a) J. P. Monserrat, G. G. Chabot, L. Hamon, L. Quentin, D. Scherman, G. Jaouen and E. A. Hillard, *Chem. Commun.*, 2010, **46**, 145; (b) D. Kumar, G. Patel, B. G. Mishra and R. S. Varma, *Tetrahedron Lett.*, 2008, **49**, 6974; (c) H. Kagawa, A. Shigematsu, S. Ohta and Y. Harigaya, *Chem. Pharm. Bull.*, 2005, **53**, 547; (d) L. Reichel and G. Proksch, *Justus Liebigs Ann. Chem.*, 1971, **745**, 59; (e) C. M. Brennan, I. Hunt, T. C. Jarvis, C. D. Johnson and P. D. McDonnell, *Can. J. Chem.*, 1990, **68**, 1780; (f) G. Wurm and D. Schnetzer, *Arch. Pharm.*, 1992, **325**, 717; (g) R. Chaturvedi, P. N. Patil and N. B. Mulchandani, *Indian J. Chem.*, 1992, **31B**, 340; (h) G. Nabaei-Bidhendi and N. R. Bannerjee, *J. Indian Chem. Soc.*, 1990, **67**, 43.
- (a) I. Akçok and A. Çağır, *Bioorg. Chem.*, 2010, **38**, 139; (b) S. Chandrasekhar, K. Vijeender and K. V. Reddy, *Tetrahedron Lett.*, 2005, **46**, 6991; (c) L. Reichel and F. G. Weber, *Die Pharmazie*, 1975, **30**, 195; (d) D. D. Keane, K. G. Marathe, W. I. O'sullivan and S. Srivastava, *J. Org. Chem.*, 1970, **35**, 2286; (e) A. Kumar, S. Sharma, V. D. Tripathi and S. Srivastava, *Tetrahedron*, 2010, **66**, 9445.
- O. V. Sing, *Org. Prep. Proced. Int.*, 1993, **25**, 693.
- (a) S. J. Joglekar and S. D. Samant, *Tetrahedron Lett.*, 1988, **29**, 241; (b) D. Kim, K. Ham and S. Hong, *Org. Biomol. Chem.*, 2012, **10**, 7305.
- See for example: (a) Y. Maki, K. Shimada, M. Sako and K. Hirota, *Tetrahedron*, 1988, **44**, 3187; (b) G. Pandey, G. Kumaraswamy and A. Krishna, *Tetrahedron Lett.*, 1987, **28**, 2649; (c) T. M. Harris and R. L. Carney, *J. Am. Chem. Soc.*, 1967, **89**, 6734.
- A. V. Dubrovskiy and R. C. Larock, *Org. Lett.*, 2010, **12**, 3117.
- (a) V. Kavala, C. Lin, C. W. Kuo, H. Fang and C. F. Yao, *Tetrahedron*, 2012, **68**, 1321; (b) H. Eshghi, M. Rahimizadeh and S. M. Mousavi, *Nat. Prod. Res.*, 2014, **28**, 438; (c) D. D. Kondhare, G. Gyananath, Y. Tamboli, S. S. Kumbhar, P. B. Choudhari, M. S. Bhatia and P. K. Zubaidha, *Med. Chem. Res.*, 2017, **26**, 987.
- (a) L. Zhang, Y. Zhao, J. Wang, D. Yang, C. Zhao, C. Wang, C. Ma and M. Cheng, *Eur. J. Med. Chem.*, 2018, **151**, 27; (b) P. Barai, N. Raval, S. Acharya and N. Acharya, *Biomed. Pharmacother.*, 2018, **108**, 374.
- (a) D. Bano and P. Nicotera, *Stroke*, 2007, **38**, 674; (b) R. Guerrini, E. Marzola, C. Trapella, S. Molinari, M. C. Cerlesi, D. Malfacini and S. Salvadori, *Bioorg. Med. Chem.*, 2014, **22**, 3703.
- L. Yang, E. Wang, Y. Fan, J. Yang, Z. Luo, Y. Wang, M. Peng, T. Deng and X. Yang, *Tetrahedron Lett.*, 2020, **61**, 151180.
- See for example: (a) H. R. Snyder and F. X. Werber, *J. Am. Chem. Soc.*, 1950, **72**, 2962; (b) C. D. Hurd and S. Hayao, *J. Am. Chem. Soc.*, 1954, **76**, 5065; (c) C. R. Hanser and J. G. Murray, *J. Am. Chem. Soc.*, 1955, **77**, 3858; (d) A. V. Aksenov, D. S. Ovcharov, N. A. Aksenov, D. A. Aksenov, O. N. Nadein and M. Rubin, *RSC Adv.*, 2017, **7**, 29927; (e) M. Chang, T. Lee and M. Wu, *Org. Lett.*, 2012, **14**, 2198; (f) A. V. Aksenov, V. Khamraev, N. A. Aksenov, N. K. Kirilov, D. A. Domenyuk, V. A. Zelensky and M. Rubin, *RSC Adv.*, 2019, **9**, 6636.
- (a) R. L. Galli, B. Shukitt-Hale, K. A. Youdim and J. A. Joseph, *Ann. N. Y. Acad. Sci.*, 2002, **959**, 128; (b) K. A. Youdim and J. A. Joseph, *Free Radical Biol. Med.*, 2001, **30**, 583; (c) K. Unno, F. Takabayashi, T. Kishido and N. Oku, *Exp. Gerontol.*, 2004, **39**, 1027; (d) S. Kuriyama, A. Hozawa,



- K. Ohmori, T. Shimazu, T. Matsui, S. Ebihara and I. Tsuji, *Am. J. Clin. Nutr.*, 2006, **83**, 355; (e) A. M. Haque, M. Hashimoto, M. Katakura, Y. Tanabe, Y. Hara and O. Shido, *J. Nutr.*, 2006, **136**, 1043.
- 19 D. Vauzour, K. Vafeiadou, C. Rice-Evans, R. J. Williams and J. P. Spencer, *J. Neurochem.*, 2007, **103**, 1355.
- 20 L. Yang, Q. Yang, E. Wang, J. Yang, Q. Li, J. Cao, L. Wang, X. Liao, Y. Yang and X. Yang, *Bioorg. Med. Chem.*, 2022, **59**, 116675.

