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Discovery and synthesis of novel luteolin derivatives as DAT agonists

Jiange Zhang [a,*], Xianbo Liu [a,b], Xinsheng Lei [b,*], Lei Wang [a], Lihe Guo [c,*], Gang Zhao [c], Guoqiang Lin [b]

[a] Department of Medicinal Chemistry, School of Pharmaceutical Science, Zhengzhou University, Zhengzhou 450001, China
[b] Department of Medicinal Chemistry, School of Pharmacy, Fudan University, 138 Yixueyuan Road, Shanghai 200032, China
[c] Cell Star Bio-Technologies Co., Ltd, 898 Halei Road, Shanghai 201203, PR China

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ABSTRACT

Luteolin, 5,7-dihydroxy-2-(3,4-dihydroxyphenyl)-4*H*-chromen-4-one, has been proposed and proved to be a novel dopamine transporter (DAT) activator. In order to develop this potential of luteolin, a series of novel luteolin derivatives were designed, synthesized, and evaluated for their DAT agonistic activities, utilizing constructed Chinese hamster ovary (CHO) cell lines stably expressing rat DAT. Biological screening results demonstrated that luteolin derivatives **1d**, **1e**, and **4c** carry great DAT agonistic potency (EC_{50} = 0.046, 0.869, and 1.375 μM, respectively) compared with **luteolin 8** (EC_{50} = 1.45 ± 0.29 μM). Luteolin derivative **1d**, notably, exhibited a 32-fold-higher DAT agonistic potency than luteolin. These luteolin derivatives represent a novel DAT agonist class, from which lead compounds useful for exploration of additional novel DAT agonists could be drawn.

1. Introduction

Psychiatric and neurological diseases compromise the health of multiple millions of people. Neurotransmitters such as dopamine (DA), noradrenaline (NA), and serotonin (5-HT), for example, have been implicated as playing key roles in psychiosis and neurogenic diseases. Dopamine, one of the most important neurotransmitters, affects brain processes, causing diseases as dopaminergic neurotransmission dysfunction in the central nervous system (CNS),[1–3] such as Parkinson's disease (PD), schizophrenia, attention-deficit hyperactivity disorder, and drug addiction.[4,5]

In response to certain physiological actions, DA is released from nerve cells into the synapses, where it binds to its receptors on the postsynaptic membrane. DA is inactivated mainly by reuptake via the dopamine transporter (DAT), the monamine transport protein on the presynaptic membrane of dopaminergic neurons existing in areas of the brain where DA signaling is common.[6] Thus, DAT plays a key role in regulating dopaminergic signaling in the brain by mediating rapid clearance of dopamine from the synaptic clefts and then it is involved in a number of DA-related disorders, including attention-deficit hyperactivity disorder, bipolar disorder, clinical depression, and alcoholism.[7,8]

In the last 20 years, several classes of DAT antagonists that were tested preclinically for their potential as treatments for stimulant abuse have been reviewed elsewhere.[9] Nonetheless, to date, no practical pharmaceutical can act as DAT agonists, which we assume would act by positively modulating DAT activity, an opposing effect of the transporter antagonists. DAT agonists, significantly, strengthen the DAT' reuptake action, thereby relieve psychiosis or neurogenic diseases caused by DA accentuation. Lihe Guo et al. were the first to propose and to prove that the DAT can be agitated by the polyphenolic plant-derived flavonoids luteolin and apigenin.[10,11] These two compounds possess actions of enhancing monoamine uptake either upon monoamine-transporter transgenic Chinese hamster ovary (CHO) cells or upon wild dopaminergic cell lines, with higher specificity for dopamine (DA) uptake than for norepinephrine (NE)- and serotonin (5-HT)-uptake. Therefore, luteolin and apigenin can be regarded as novel monoamine-transporter activators. However, the DAT agonistic potency and efficacy of luteolin are stronger than that of apigenin. Luteolin was thereby chosen as the candidate compound to be used in our study.

In the present study, luteolin was chosen as lead compounds for exploration of novel compounds with more DAT agonistic activities and better water-solubility. A series of novel luteolin derivatives, 5-acylluteolin derivatives **1a–i**, 5-alkylluteolin derivatives **2a–k**, 7-alkylluteolin derivatives **3a–e**, 7-acylluteolin derivatives **4a–c**, 5,7-dimethylluteolin derivative **5a**, 3′,4′-dialkylluteolin derivative **5b**, 4′-alkylluteolin derivatives **6a,b** and 3′-alkylluteolin derivatives 7a,**b**, thus were synthesized and screened using a transgenic CHO (Tr-CHO) cell-line system and discovered that luteolin derivatives **1d**, **1e**, and **4c** exhibited more DAT agonistic activities than luteolin, especially, compound **1d** exhibited a 32-fold-higher DAT agonistic potency than luteolin.

* Corresponding authors. Tel.: +86 371 6778 1908 (J.Z.); tel.: +86 21 51980128 (X.L.).
E-mail addresses: jgzhang5500@hotmail.com (J. Zhang), leixs@fudan.edu.cn (X. Lei), lhguo@sibs.ac.cn (L. Guo).

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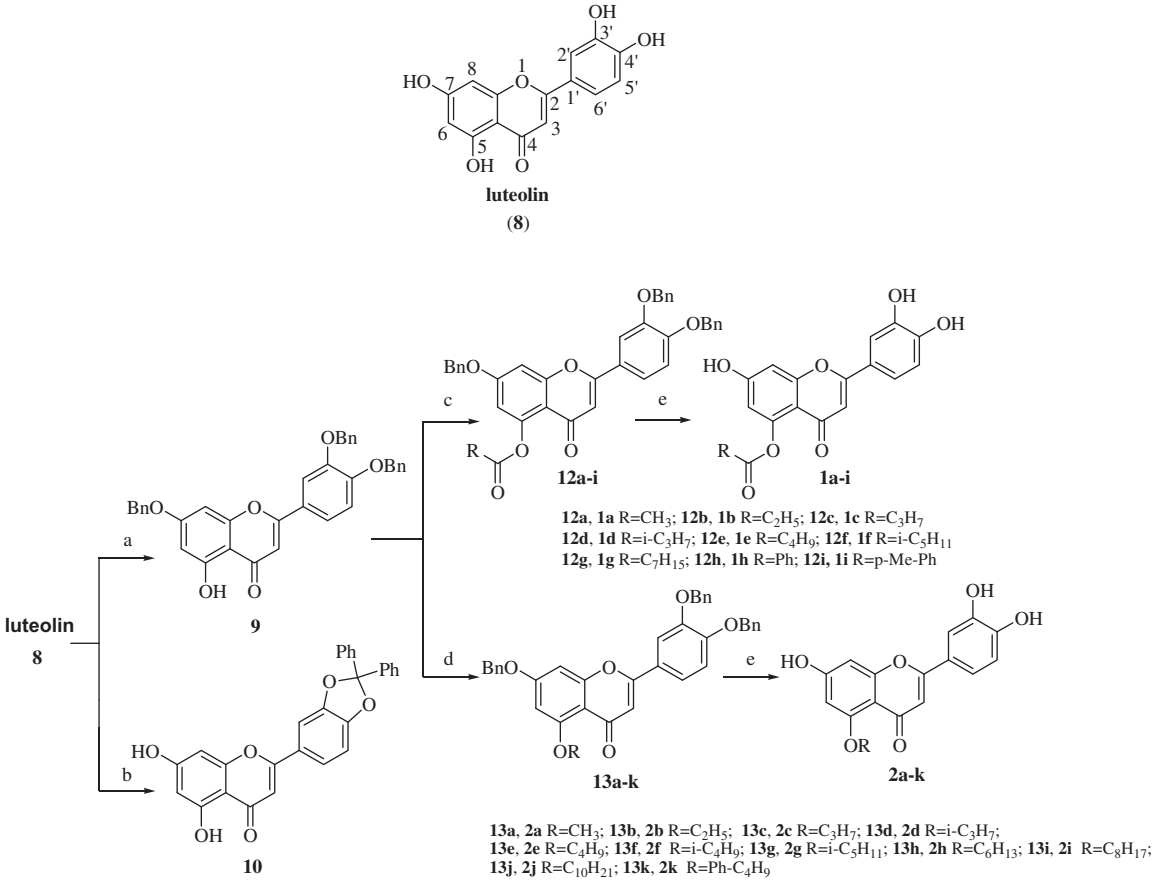
2. Chemistry

Since the structure of luteolin **8** contains polyphenolic groups, and its four phenolic hydroxyls posses different reactivities, for example, in the acetylation reaction, the order from strong to weak is 4′-, 7->3′->5-, hence, addition of different substituent groups at certain positions on the luteolin ring is feasible. 5-Acylluteolin derivatives **1a–i** and 5-etherluteolin derivatives **2a–k** were prepared according to Scheme 1. The key intermediate **9** was synthesized by treatment of luteolin **8** with 3 equiv amounts of benzyl bromide and K_2CO_3 in dimethyl formamide (DMF). Compounds **12a–i** were synthesized by esterification of **9** with excess respective acyl chlorides and NaH in CH_2Cl_2 or with the carboxylic acids, DCC, and DMAP in CH_2Cl_2 at room temperature. These syntheses were accomplished initially under various conditions such as K_2CO_3/DMF, pyridine/CH_2Cl_2, concd sulphuric acid/CH_2Cl_2, and others, but the resulting yields were poor, due to the intramolecular hydrogen bond between carbonyl group of C4 and adjacent hydroxyl of group of C5 of flavonoid.[12] Subsequently, the benzyl protection groups in **12a–i** were removed by transfer hydrogenolysis using palladium hydroxide (on carbon, containing 20% pd) in EtOH–THF at rt, thereby yielding the target compounds **1a–i**. Similarly, compound **9** was etherified by treatment of substituted halogenated hydrocarbon with K_2CO_3, affording to produce **13a–k**. The 5-alkylluteolin derivatives **2a–k** were obtained by debenzylation of **13a–k** under the same conditions.

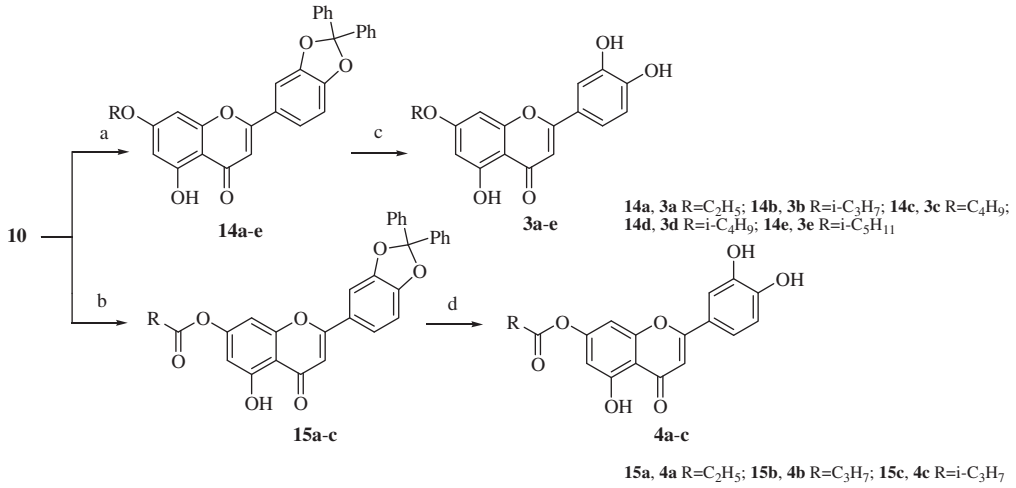
The syntheses of the 7-etherluteolin derivatives **3a–e** and the 7-acylluteolin derivatives **4a–c** are shown in Scheme 2. Luteolin **8** was heated with dichlorodiphenylmethane at 180 °C for 20 min to produce compound **10**. Displacement of the 7-hydrogen atom in **10** with the respective halogenated hydrocarbons and K_2CO_3 provided compounds **14a–e**. Cleavage of the dibenzal groups of **14a–e** with a mixture of acetic acid and water (4:1) gave the corresponding 7-alkylluteolin derivatives **3a–e**. The requisite esters **4a–c** were prepared by condensation of **10** with the equivalent respective acyl chlorides and KOH or K_2CO_3, followed by the hydrogenolysis of dibenzal groups of **15a–c** with palladium hydroxide in EtOH–THF at rt, producing good yields. However, the dibenzal groups of **15a–c** were partially hydrogenised if they were treated with a mixture of acetic acid and water (4:1).

Compound **10** was treated with 2 equiv MeI and K_2CO_3 to produce 5,7-dimethylluteolin derivative **16**. Subsequently, 5,7-dimethylluteolin derivative **5a** was synthesized by removal of the dibenzal group with a mixture of acetic acid and water (4:1). The corresponding displacement reaction using 2-iodopropane or 1-iodo-2-methylpropane instead of MeI did not proceed, perhaps because slightly large groups can cause steric hindrance, only 5,7-dimethylluteolin derivative **5a** was obtained (Scheme 3).

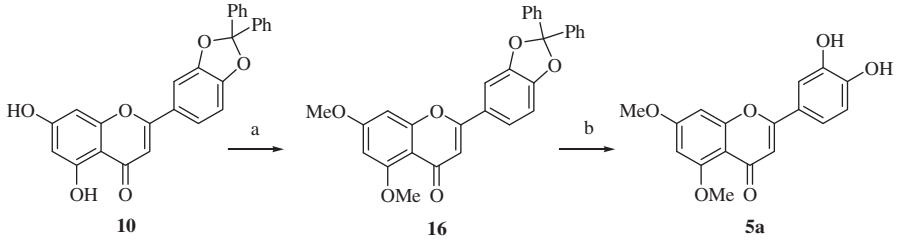
By Scheme 4, the 4′-alkylluteolin derivatives **6a,b** were synthesized. Protection of the 5- and 7-hydroxyl groups of compound **10** with benzyl bromide and K_2CO_3 in DMF produced **17**, in excellent yield. Selective removal of the 5-benzyl group and the dibenzal group from **17** by means of the acetic acid and water mixture (4:1) at reflux afforded **19** in 96.8% yield, in which the 4′-hydroxyl group was displaced using 1 equiv corresponding halogenated hydrocarbon and K_2CO_3 in DMF. Finally, compounds **6a,b** were prepared by hydrogenolysis with palladium hydroxide. Also, both the 3′- and 4′-hydroxyl groups in **19** were displaced using 2 equiv C_2H_5Br and K_2CO_3 in DMF, yielding compound **21**. Finally, the 3′,4′-diethylluteolin derivative **5b** was produced in 71% yield by hydrogenolysis of **21** on palladium hydroxide (Scheme 5).



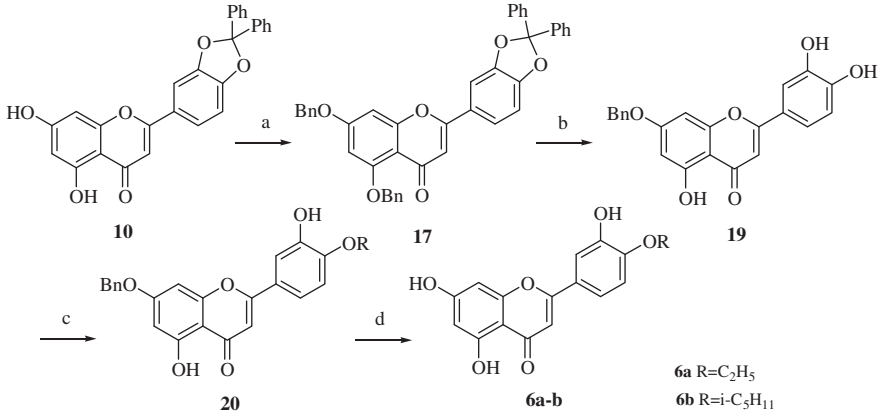
Scheme 1. Reagents and conditions: (a) BnBr/K_2CO_3/DMF; (b) Ph_2CCl_2, 180 °C; (c) RCOCl/NaH/CH_2Cl_2 or RCOOH/DMAP/DCC/CH_2Cl_2, rt; (d) R′X/K_2CO_3/DMF, rt–70 °C, X = I or Br; (e) H_2/20% $Pd(OH)_2$/C/THF/EtOH.



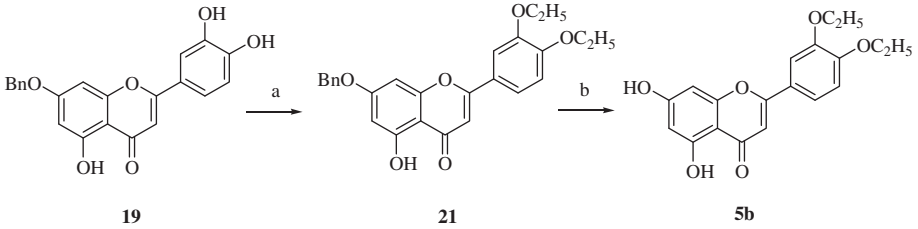
Scheme 2. Reagents and conditions: (a) RX/K_2CO_3/DMF, 70 °C, X = I or Br; (b) R′COCl/KOH or K_2CO_3/DMF, rt; (c) AcOH/H_2O (4:1), reflux; (d) H_2/20% $Pd(OH)_2$/C/THF/EtOH.



Scheme 3. Reagents and conditions: (a) MeI/K_2CO_3/DMF, rt; (b) AcOH/H_2O (4:1), reflux.

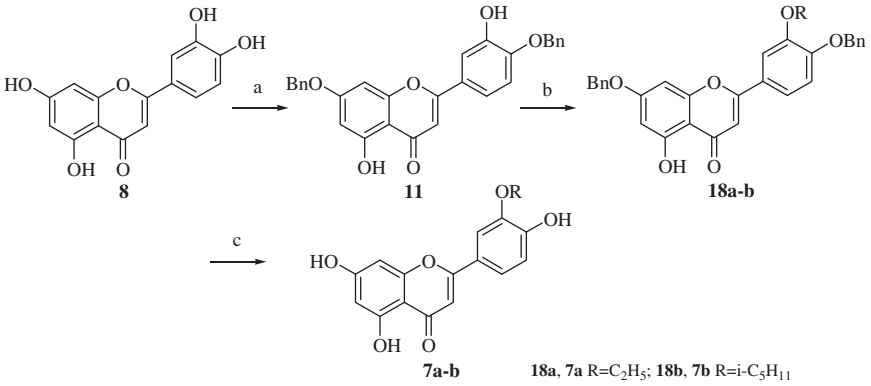


Scheme 4. Reagents and conditions: (a) BnBr/K_2CO_3/DMF; (b) AcOH/H_2O (4:1), reflux; (c) 1 equiv RX/K_2CO_3/DMF; (d) H_2/20% $Pd(OH)_2$/C/THF/EtOH X = I or Br.



Scheme 5. Reagents and conditions: (a) 2 equiv C_2H_5Br/K_2CO_3/DMF; (b) H_2/20% $Pd(OH)_2$/C/THF/EtOH.

Reaction of luteolin **8** with 2 equivalent of benzyl bromide afforded the main product **11** (Scheme 6), in which the 7- and 4′-hydroxyl groups were protected by the benzyl group. Displacement of the 3′-hydrogen atom in **11** by halogenated hydrocarbon



Scheme 6. Reagents and conditions: (a) 2 equiv BnBr/K_2CO_3/DMF; (b) RX/K_2CO_3/DMF; (c) H_2/20% $Pd(OH)_2$/C/THF/EtOH X = I or Br.

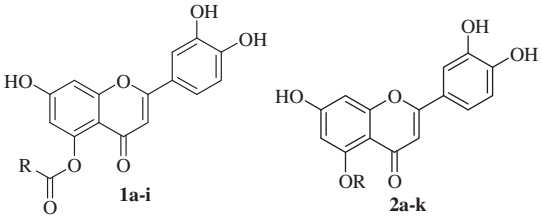
and K_2CO_3 resulted in compounds **18a,b**. The target-compound 3′-alkylluteolin derivatives **7a,b** were produced in good yield by hydrogenolysis on palladium hydroxide.

3. Results and discussion

All of the synthesized luteolin derivatives and the three intermediates (**10**, **11**, and **19**), in comparison with luteolin **8** (EC_{50} = 1.45 ± 0.29 μM), were evaluated in vitro for DAT agonistic activities (expressed in EC_{50} values). First, 5-acylluteolin derivatives **1a–i** and 5-alkylluteolin derivatives **2a–k** were synthesized and evaluated for those activities by means of constructed CHO cell lines. Table 1 lists the results obtained. 5-isobutyrylluteolin derivative **1d** (EC_{50} = 0.046 μM) was found to be the most potent DAT agonist among the luteolin derivatives, exhibiting a 32-fold-higher DAT agonistic potency than luteolin **8**. 5-Pentanoylluteolin derivative **1e**, as compared with the latter, likewise revealed a higher level of DAT agonistic activity (EC_{50} = 0.869 μM). 5-Butyrylluteolin derivative **1c** and 5-iso hexanoylluteolin derivative **1f**, meanwhile, showed an agonistic potency approximately equal to that of luteolin **8**. The DAT agonistic activity decreased as a result of 5-acyl substitution for the acetyl group, the propionyl group, the benzoyl group, and the 4-methylbenzoyl group in **1a**, **1b**, **1g**, **1h**, and **1i**, the EC_{50} values falling in the 5.368 – 30.978 μM range. This fact suggests that the various 5-acyl substituents of luteolin **8** are essential to DAT agonistic activity and that hydrocarbon substituent (in possession of 4–6 carbons) is integral to that activity. However, the EC_{50} results for 5-alkylluteolin derivatives **2a–k** indicated a lesser DAT agonistic potency than that of luteolin **8** regardless of the 5-alkyl substituent. This suggests that substitution of an acyl group for the 5-hydrogen atom in luteolin **8** is more effective than substitution of an alkyl group.

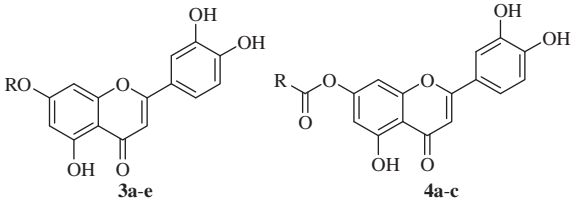
The discovery of 7-alkylluteolin derivatives **3a–e** and 7-acylluteolin derivatives **4a–c** led us to explore C-7 modification on the luteolin ring, as a means of further improving DAT agonistic potency (Table 2). All of the synthesized 7-alkylluteolin derivatives **3a–e** and the intermediate **19** were inactive. The DAT agonistic activities of 7-acylluteolin derivatives **4a**, **4b**, and **4c** were slightly reduced or equipotent compared with luteolin **8**. This suggests, further, that the C-5 or C-7 ester derivatives of luteolin are more favorable than the C-5 or C-7 ether derivatives of same.

Table 1
In vitro DAT agonistic activities of **1a**–**i** and **2a**–**k**



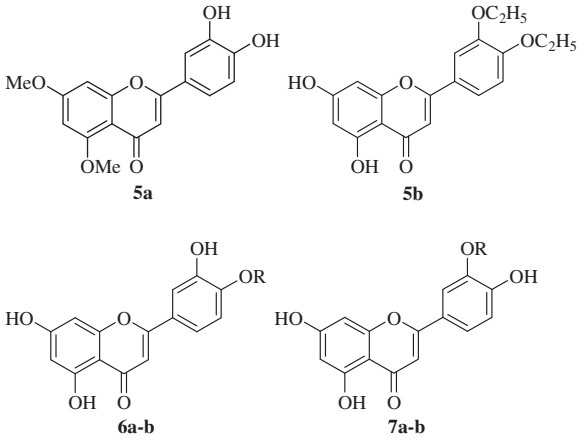
Compound	R	EC_{50} (μM)
1a	CH_3	5.368
1b	C_2H_5	30.978
1c	C_3H_7	1.820
1d	*i*-C_3H_7	**0.046**
1e	C_4H_9	**0.869**
1f	*i*-C_5H_{11}	2.706
1g	C_7H_{15}	8.557
1h	Ph	11.174
1i	*p*-Me–Ph	6.920
2a	CH_3	4.345
2b	C_2H_5	2.213
2c	C_3H_7	3.072
2d	*i*-C_3H_7	5.430
2e	C_4H_9	2.40192
2f	*i*-C_4H_9	4.798
2g	*i*-C_5H_{11}	3.428
2h	C_6H_{13}	4.575
2i	C_8H_{17}	16.521
2j	$C_{10}H_{21}$	33.002
2k	Ph–C_4H_8	2.594
8		1.45 ± 0.29

Table 2
In vitro DAT agonistic activities of **3a**–**e** and **4a**–**c**



Compound	R	EC_{50} (μM)
3a	C_2H_5	–
3b	*i*-C_3H_7	–
3c	C_4H_9	–
3d	*i*-C_4H_9	–
3e	*i*-C_5H_{11}	–
19	Ph–CH_2	–
4a	C_2H_5	1.696
4b	C_3H_7	1.694
4c	*i*-C_3H_7	**1.375**
8		1.45 ± 0.29

Table 3
In vitro DAT agonistic activities of **5a**, **5b**, **6a**,**b**, **7a**,**b** and **10**, **11**



Compound	R	EC_{50} (μM)
5a	—	3.417
5b	—	—
6a	C_2H_5	—
6b	*i*-C_5H_{11}	—
7a	C_2H_5	12.856
7b	*i*-C_5H_{11}	—
10		—
11		—
8		1.45 ± 0.29

Comparing the 5,7-dimethylluteolin derivative **5a**, the 3′,4′-diehtylluteolin derivative **5b**, the 4′-alkylluteolin derivatives **6a,b**, the 3′-alkylluteolin derivatives **7a,b** and the intermediates **10** and **11** as shown in Table 3, **5a** showed reduced DAT agonistic activity. The fact that the 3′-, or the 4′-, or the 3′,4′-substituted derivatives of luteolin **8** showed lesser activity or inactivity implies that the free hydroxyl at 3′- and 4′- is essential to DAT agonistic functionality. It is possible that introduction of a substituent at the 3′- or 4′-position on the luteolin ring prevents combination with the DAT, thereby reducing agonistic potency.

4. Conclusions

It was found that the optimal DAT agonistic structure features of luteolin **8** are: (1) the number and type of acyl substituents at C-5 or C-7, hydrocarbon substituent (in possession of 4–6 carbons) being crucial and (2) the free hydroxyl at 3′- and 4′-. These findings led to the discovery of 5-isobutyrylluteolin derivative **1d** as a potent DAT agonist. Compound **1d** offers a 32-fold increase in potency over that of the parent core **8**. Further, in vivo studies on compound **1d** are ongoing.

5. Experimental

Materials were obtained from commercial suppliers. ^{1}H NMR spectra were obtained on NMR spectrometer with superconductor magnet (MERCURY 300). The chemical shifts are given in δ values (ppm) using tetramethylsilane as the internal standard. ESI-MS were determined on LCMS-2010EV spectrometer. MALDI-MS were determined on Ion Spec HiResMALDI spectrometer. IR was recorded on FT-185 spectrometer. HRMS were determined on Ion Spec 4.7 Tesla FTMS. Reactions were followed by TLC on Silica gel (HSGF 254). Chromatographic separations were carried out on silica gel (300–400 mesh) using the indicated eluents. Yields are unoptimized. Chemical intermediates were characterized by ^{1}H NMR.

5.1. 7-(Benzyloxy)-2-(3′,4′-bis(benzyloxy)phenyl)-5-hydroxy-4*H*-chromen-4-one 9

A mixture of luteolin **8** (0.1 g, 0.35 mmol), benzyl bromide (0.125 mL, 1.05 mmol), and anhydrous K_2CO_3 (0.145 g, 1.05 mmol) in DMF (10 mL) was stirred at 0 °C to rt overnight. The reaction mixture was diluted with EtOAc and washed with water. The organic layer was separated, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography (2:1 petroleum/ether to 1:1 petroleum/CH_2Cl_2) to give stramineous solid product **9** (0.109 g, 56%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.084 (s, 2H), 5.202 (s, 4H), 6.391–6.399 (d, 1H, J = 2.4 Hz), 6.450 (s, 1H), 6.475–6.481 (d, 1H, J = 1.8 Hz), 6.949–6.978 (d, 1H, J = 8.7 Hz), 7.312–7.485 (m, 17H), 12.787 (s, 1H); MS (ESI, m/z): 557.5 [M+H$^+$], 579.6 [M+Na$^+$].

5.2. 2-(2,2-Diphenylbenzo[*d*][1,3]dioxol-5-yl)-5,7-dihydroxy-4*H*-chromen-4-one 10

A mixture of luteolin **8** (2 g, 6.99 mmol) and dichlorodiphenylmethane (2 mL,10.42 mmol) was intimately mixed then heated at 180 °C for 20 min. till the white gas did not emit. The crude resulting products was diluted with hot acetone then the insoluble substance was strained off. The filtrate obtained was concentrated then purified by flash column chromatography using petroleum ether/EtOAc (6:1 to 1:1) as eluent, vacuum dehydration to give yellow solid product **10**: 1.248 g, 39.6% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.177–6.184 (d, 1H, J = 2.1 Hz), 6.499–6.506 (d, 1H, J = 2.1 Hz), 6.877 (s, 1H), 7.198–7.226 (d, 1H, J = 8.4 Hz), 7.438–7.539 (m, 10H), 7.679–7.707 (d, 1H, J = 8.4 Hz), 7.772 (s, 1H), 10.884 (s, 1H), 12.856 (s, 1H); MS (ESI, m/z): 449.1 [M−H$^-$].

5.3. 7-(Benzyloxy)-2-(3,4-bis(benzyloxy)phenyl)-4-oxo-4*H*-chromen-5-yl acetate 12a

A solution of **9** (300 mg, 0.54 mmol) in DMF (10 mL), 60% NaH (44 mg, 1.1 mmol) was added under argon, stirred for 30 min. Acetyl chloride (77 μL, 1.08 mmol) was added. After stirring at rt for 2 h, the resulting mixture was diluted with EtOAc, and then according to the general disposal procedure, crude products was purified by flash column chromatography using petroleum ether/EtOAc (6:1 to 1:1) as eluent to afford stramineous solid product **12a**: 320 mg, 99% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.430 (s, 3H), 5.142 (s, 2H), 5.233 (s, 4H), 6.433 (s, 1H), 6.669–6.677 (d, 1H, J = 2.4 Hz), 6.880–6.888 (d, 1H, J = 2.4 Hz), 6.974–7.002 (d, 1H, J = 8.4 Hz), 7.323–7.487 (m, 17H); MS (MALDI, m/z): 599.2 [M+H$^+$], 621.2 [M+Na$^+$]. Compounds **12b–i** was prepared as described for compound **12a** (Supplementary data).

5.4. 2-(3,4-Dihydroxyphenyl)-7-hydroxy-4-oxo-4*H*-chromen-5-yl acetate 1a

A suspension of **12a** (598 mg, 0.535 mmol) in EtOH (15 mL) was treated with 20% palladium hydroxide (32 mg) under a flow of hydrogen for 7 h at rt. The reaction mixture was then filtered on Celite and eluted with EtOH. After concentration of the filtrate under vacuum, EtOAc was added to collect product then hexane was added to scorify solid. Solid was filtered, washed with hexane and vacuum drying to give flavovirens solid product **1a**: 150 mg, 85.4% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.285 (s, 3H), 6.462 (s, 1H), 6.530–6.538 (d, 1H, J = 2.4 Hz), 6.860–6.868 (d, 1H, J = 2.4 Hz), 6.900 (s, 1H), 7.355 (s, 1H), 7.377 (s, 1H); MS (ESI, m/z): 327.0 [M−H$^-$], 284.9 [M−CH_3CO^-]; FT-IR: ν_{max} 3375 (br, OH), 2924, 2854, 1744, 1658, 1615, 1494, 1450, 1367, 1257, 1166, 946, 830 cm^{-1}; HRMS (MALDI) m/z calcd for $C_{17}H_{13}O_7$ [M+H$^+$]: 329.0650, found 329.06558. Com-

pounds **1b–i** was prepared as described for compound **1a** (Supplementary data).

5.5. 7-(Benzyloxy)-2-(3,4-bis(benzyloxy)phenyl)-5-ethoxy-4*H*-chromen-4-one 13b

A solution of **9** (300 mg, 0.54 mmol) in DMF (10 mL), anhydrous potassium carbonate (149 mg, 1.08 mmol), and iodoethane (87 μL, 1.08 mmol) were added under argon. After stirring at 75 °C for 12 h, the resulting mixture was diluted with EtOAc, and then according to the general disposal procedure, crude products was purified by flash column chromatography using petroleum ether/EtOAc (6:1 to 3:1) as eluent to afford stramineous solid product **13b**: 311 mg, 98.7% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.508–1.554 (t, 3H, J = 6.9 Hz), 4.084–4.155 (q, 2H, J = 6.9 Hz), 5.125 (s, 2H), 5.220 (s, 4H), 6.413–6.421 (d, 1H, J = 2.4 Hz), 6.492 (s, 1H), 6.559–6.567 (d, 1H, J = 2.4 Hz), 6.967–6.995 (d, 1H, J = 8.4 Hz), 7.315–7.502 (m, 17H); MS (MALDI, m/z): 585.4 [M+H$^+$], 607.4 [M+Na$^+$]. Compounds **13a** and **13c–k** were prepared as described for compound **13b** (Supplementary data).

5.6. 2-(3,4-Dihydroxyphenyl)-5-ethoxy-7-hydroxyl-4*H*-chromen-4-one 2b

From compound **13b** (100 mg, 0.17 mmol) as described for **1a**, flavovirens solid product **2b** was obtained: 30 mg, 57% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.314–1.360 (t, 3H, J = 6.9 Hz), 3.976–4.046 (q, 2H, J = 6.9 Hz), 6.327 (s, 1H), 6.352 (s, 1H), 6.455 (s, 1H), 6.830–6.858 (d, 1H, J = 8.4 Hz), 7.290–7.296 (d, 2H, J = 1.8 Hz); MS (ESI, m/z): 313.1 [M−H$^-$]; FT-IR: ν_{max} 3472 (br, OH), 2950, 1604, 1520, 1465, 1374, 1277, 1114, 1025, 825, 786 cm^{-1}; HRMS (MALDI) m/z calcd for $C_{17}H_{15}O_6$ [M+H$^+$]: 315.0864, found 315.08632. Compounds **2a** and **2c–k** were prepared as described for compound **2b** (Supplementary data).

5.7. 2-(2,2-Diphenylbenzo[*d*][1,3]dioxol-5-yl)-7-ethoxy-5-hydroxy-4*H*-chromen-4-one 14a

From compound **10** (300 mg, 0.67 mmol) and iodoethane (54 μL, 0.67 mmol) as described for **13b** to obtain yellow solid product **14a**: 219 mg, 69% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.425–1.472 (t, 3H, J = 6.9 Hz), 4.060–4.130 (q, 2H, J = 6.9 Hz), 6.339–6.346 (d, 1H, J = 2.1 Hz), 6.437–6.444 (d, 1H, J = 2.1 Hz), 6.519 (s, 1H), 6.975–7.003 (d, 1H, J = 8.4 Hz), 7.389–7.469 (m, 8H), 7.750–7.600 (m, 4H), 12.754 (s, 1H); MS (ESI, m/z): 479.1 [M+H$^+$], 501.2 [M+Na$^+$]. Compounds **14b–e** was prepared as described for compound **14a** (Supplementary data).

5.8. 2-(3,4-Dihydroxyphenyl)-7-ethoxy-5-hydroxy-4*H*-chromen-4-one 3a

Compound **14a** (219 mg, 0.458 mmol) was added to a mixture of acetic acid/water (4:1, 50 mL) and refluxed for 9 h. Then, EtOAc (100 mL) and water (100 mL) were added. The organic layer was washed with $NaHCO_3$ saturated aqueous solution (40 mL × 3) and dried over anhydrous Na_2SO_4. After concentration, the residue was added with copious dichloromethane and petroleum ether. Solid scorified was filtered, washed with petroleum ether, and vacuum drying to give yellow solid product **3a**: 80 mg, 56% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.335–1.381 (t, 3H, J = 6.9 Hz), 4.120–4.190 (q, 2H, J = 6.9 Hz), 6.345–6.352 (d, 1H, J = 2.1 Hz), 6.703–6.710 (d, 1H, J = 2.1 Hz), 6.728 (s, 1H), 6.886–6.913 (d, 1H, J = 8.1 Hz), 7.434–7.461 (d, 1H, J = 8.1 Hz), 7.467 (s, 1H), 12.986 (s, 1H); MS (ESI, m/z): 313.0 [M−H$^-$]; FT-IR: ν_{max} 3415 (br, OH), 2750, 1656, 1500, 1419, 1336, 1245, 1125, 1032, 859, 763, 686 cm^{-1}; HRMS (MALDI) m/z calcd for $C_{17}H_{15}O_6$ [M+H$^+$]: 315.0858, found 315.08632. Compounds **3b–e** was prepared as described for compound **3a** (Supplementary data).

5.9. 2-(2,2-Diphenylbenzo[*d*][1,3]dioxol-5-yl)-5-hydroxy-4-oxo-4*H*-chromen-7-yl propionate 15a

Compound **10** (300 mg, 0.67 mmol) in DMF (15 mL), anhydrous potassium carbonate (185 mg, 0.84 mmol) was added under argon, stirred for 30 min. Propionyl chloride (57 μL, 0.67 mmol) was added. After vigorous stirring at rt for 24 h, the resulting mixture was diluted with dichloromethane, and then according to the general disposal procedure, crude products was purified by flash column chromatography using dichloromethane as eluent to afford yellow solid product **15a**: 251 mg, 74.4% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.258–1.309 (t, 3H, J = 7.5 Hz), 2.586–2.661 (q, 2H, J = 7.5 Hz), 6.549–6.556 (d, 1H, J = 2.1 Hz), 6.584 (s, 1H), 6.806–6.813 (d, 1H, J = 2.1 Hz), 6.979–7.007 (d, 1H, J = 8.4 Hz), 7.380–7.480 (m, 8H), 7.578–7.610 (m, 4H), 12.788 (s, 1H). Compounds **15b,c** was prepared as described for compound **15a** (Supplementary data).

5.10. 2-(3,4-Dihydroxyphenyl)-5-hydroxy-4-oxo-4*H*-chromen-7-yl propionate 4a

From compound **15a** (185 mg) as described for **1a**, flavovirens solid product **4a** was obtained: 86 mg, 69% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.128–1.176 (t, 3H, J = 7.2 Hz), 2.609–2.683 (q, 2H, J = 7.5 Hz), 6.636–6.642 (d, 1H, J = 1.8 Hz), 6.852 (s, 1H), 6.900–6.928 (d, 1H, J = 8.4 Hz), 7.033–7.039 (d, 1H, J = 1.8 Hz), 7.456 (s, 1H), 7.464–7.492 (d, 1H, J = 8.4 Hz), 13.057 (s, 1H); MS (ESI, m/z): 341.1 [M−H$^-$]; FT-IR: ν_{max} 3400 (br, OH), 2984, 1767, 1656, 1567, 1455, 1335, 1218, 1137, 1074, 944, 878, 740, 639 cm^{-1}; HRMS (MALDI) m/z calcd for $C_{18}H_{15}O_7$ [M+H$^+$]: 343.0815, found 343.08123. Compounds **4b,c** was prepared as described for compound **4a** (Supplementary data).

5.11. 2-(3,4-Dihydroxyphenyl)-5,7-dimethoxy-4*H*-chromen-4-one 5a

From compound **10** (300 mg, 0.67 mmol) and iodomethane (167 μL, 2.68 mmol) as described for **13b** to obtain white solid product **16**: 280 mg, 88% yield. Then, from compound **16** (280 mg, 0.59 mmol) as described for **3a**, gray solid product **5a** was obtained: 45 mg, 25% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.798 (s, 3H), 3.872 (s, 3H), 6.443 (s, 1H), 6.466–6.473 (d, 1H, J = 2.1 Hz), 6.750–6.757 (d, 2H, J = 2.1 Hz), 6.835–6.864 (d, 1H, J = 8.7 Hz), 7.328 (s, 1H), 7.350 (s, 1H); MS (ESI, m/z): 313.1 [M−H$^-$]; FT-IR: ν_{max} 3460 (br, OH), 2945, 1640, 1560, 1399, 1276, 1163, 1057, 957, 826, 741, 670 cm^{-1}; HRMS (MALDI) m/z calcd for $C_{17}H_{15}O_6$ [M+H$^+$]: 315.0863, found 315.08632.

5.12. 2-(4-Ethoxy-3-hydroxyphenyl)-5,7-dihydroxy-4*H*-chromen-4-one 6a

From compound **10** (2.26 g, 5 mmol) and benzyl bromide (1.5 mL, 12.5 mmol) as described for **15a** to afford off-white solid product **17**: 2.304 g, 73% yield. Then, compound **17** (838 mg, 1.33 mmol) was added to a mixture of acetic acid/water (4:1, 50 mL) and as described for **3a** to obtain yellow solid product **19**: 484 mg, 96.8% yield. From compound **19** (377 mg, 1 mmol) and iodoethane (80 μL, 1 mmol) as described for **13b** to obtain yellow solid product **20a**: 305 mg, 75.3% yield. Finally, from compound **20a** (293 mg) as described for **1a**, flavovirens solid product **6a** was obtained: 174 mg, 76.4% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.290–1.334 (t, 3H, J = 6.6 Hz), 4.031–4.098 (q, 2H, J = 6.6 Hz), 6.141 (s, 1H), 6.405 (s, 1H), 6.681 (s, 1H), 6.989–7.017 (d, 1H,

J = 8.4 Hz), 7.379 (s, 1H), 7.443–7.471 (d, 1H, J = 8.4 Hz), 12.885 (s, 1H); MS (ESI, m/z): 313.1 [M−H$^-$]; FT-IR: ν_{max} 3546 (br, OH), 2990, 1654, 1586, 1431, 1363, 1263, 1172, 1031, 921, 846, 799, 643 cm^{-1}; HRMS (MALDI) m/z calcd for $C_{17}H_{15}O_6$ [M+H$^+$]: 315.0869, found 315.08632. Compounds **6b** was prepared as described for compound **6a** (Supplementary data).

5.13. 2-(3,4-Diethoxyphenyl)-5,7-dihydroxy-4*H*-chromen-4-one 5b

From compound **19** (377 mg, 1 mmol) and iodoethane (160 μL, 2 mmol) as described for **13b** to obtain yellow solid product **21**: 351 mg, 81% yield. Then, from compound **21** (342 mg) as described for **1a**, flavovirens solid product **5b** (167 mg) was obtained. By flash column chromatography using petroleum ether/EtOAc (6:1) as eluent, another **5b** (24 mg) was afforded from the filtrate residue. The total **5b** was afforded: 191 mg, 71% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.285–1.332 (t, 6H, J = 7.2 Hz), 4.030–4.131 (m, 4H), 6.145–6.151 (d, 1H, J = 1.8 Hz), 6.457–6.463 (d, 1H, J = 1.8 Hz), 6.877 (s, 1H), 7.028–7.057 (d, 1H, J = 8.7 Hz), 7.483–7.489 (d, 1H, J = 1.8 Hz), 7.561–7.596 (dd, 1H, J = 1.8 Hz, J = 8.4 Hz), 12.885 (s, 1H); MS (ESI, m/z): 341.2 [M−H$^-$]; FT-IR: ν_{max} 3100 (br, OH), 2621, 1651, 1525, 1434, 1371, 1265, 1253, 1170, 1045, 1033, 905, 828, 752, 611 cm^{-1}; HRMS (MALDI) m/z calcd for $C_{19}H_{19}O_6$ [M+H$^+$]: 343.1183, found 343.11762.

5.14. 5,7-Dihydroxy-2-(4-hydroxy-3-(isopentyloxy) phenyl)-4*H*-chromen-4-one 7b

Luteolin **8** (2.87 g, 10 mmol) and benzyl bromide (2.4 mL, 20 mmol) was as described for **13b** to obtain flavovirens solid product **11** (1.913 g, 41% yield). Then, from compound **11** (268 mg, 0.575 mmol) and 1-bromo-3-methylbutane (69 μL, 0.575 mmol) as described for **13b** to afford off-white solid product **18b**: 121 mg, 39% yield. Finally, from compound **18b** (108 mg) as described for **1a**, flavovirens solid product **7b** was obtained: 70 mg, 97.6% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.906 (s, 3H), 0.928 (s, 3H), 1.582–1.649 (m, 2H), 1.729–1.859 (m, 2H), 4.084–4.091 (q, 2H, J = 6.6 Hz), 6.133–6.139 (d, 1H, J = 1.8 Hz), 6.443–6.449 (d, 1H, J = 1.8 Hz), 6.856 (s, 1H), 6.886–6.913 (d, 1H, J = 8.1 Hz), 7.490 (s, 1H), 7.515 (s, 1H), 12.932 (s, 1H); MS (ESI, m/z): 355.1 [M−H$^-$]; FT-IR: ν_{max} 3250 (br, OH), 2957, 1647, 1561, 1434, 1360, 1255, 1117, 1029, 948, 831, 724, 640 cm^{-1}; HRMS (MALDI) m/z calcd for $C_{20}H_{21}O_6$ [M+H$^+$]: 357.1333, found 357.13327. Compounds **7a** was prepared as described for compound **7b** (Supplementary data).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.09.049.

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