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Flavonoids and Biflavonoids of Amentoflavone Class as Potential Psychoactive Drug Leads

Abeer Moawad[1*] and Mohamed Hifnawy[2]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Beni-Suef University, Beni-Suef, 62514, Egypt.
[2]Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo, Egypt.

Authors' contributions

This work was carried out in collaboration between both authors. Authors AM and MH designed the study, managed the literature searches and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: There are several reported interactions between amentoflavone and CNS receptors especially GABA receptors and also reported interaction between flavonoids and opioid receptors. The current study determines other related compounds of amentoflavone, hinokiflavone and other flavonoid monomers with potential CNS activity.
Study Design: Natural and semisynthetic derivative of biflavonoids of amentoflavone and hinokiflavone class and several flavonoid monomers were screened for their binding ability to CNS receptor and neurotransmitter transporters using the Psychoactive Drug Screening Program (PDSP) University of North Carolina at Chapel Hill.
Methodology: Natural and semisynthetic flavonoid derivatives were subjected to binding assays with 44 receptors and transporters. Only compounds showing ≥50% binding inhibition in the primary assay were subjected to secondary binding assay.
Results: In the secondary binding assay; significant binding with rat benzodiazepine receptor, dopamine transporter, GABAA, norepinephrine transporter and Sigma 2 receptors were observed. (+) Catechin and sakurantin showed significant binding with dopamine transporter (K_i= 1 and 1.6

**Corresponding author: E-mail: abeermoawad77@gmail.com, abeer.moawad@pharm.bsu.edu.eg, abeer.2132006@yahoo.com;*

nM respectively) compared to the positive control (GBR 12909; K_i= 1 nM) in addition to the biflavonoid 7,7″,4‴-trimethyl-2,3-dihydroamentoflavone (16517) which showed activity at K_i= 172 nM and we present the first report of its ^{13}C NMR data. Semi synthesis afforded the new derivative 7,7″,4‴-trimethyldihydrohinokiflavone but it was inactive towards the screened receptors and neurotransmitter transporters.

Conclusion: Studying the structure-activity relationship revealed that methylation of amentoflavone decreased their ability to bind with rBZP, GABAA receptor and NET except for 7,7″,4‴-trimethyl-2,3-dihydroamentoflavone which was the most active among biflavonoids toward DAT. On the other hand, methylation of naringenin created new binding capability of sakurantin. Configurations of the chiral center at C-3 and hydroxylation pattern at ring B in flavan-3-ols greatly affect binding with dopamine transporter. Dihydrohinokiflavone and its trimethyl derivatives were completely inactive. Our study reveals new biological activity of some common flavonoids that may be promising drugs leads.

Keywords: CNS; catechin; dopamine transporter; sakurantin; amentofavone; NMR.

ABBREVIATIONS

ADHD	:	*Attention Deficit Hyperactivity Disorder*
CNS	:	*Central Nervous System*
D	:	*Dopamine*
DAT	:	*Dopamine Transporter*
DOR	:	*Delta Opioid Receptor*
GABA	:	*Gamma-Aminobutyric Acid*
H	:	*Histamine*
HMBC	:	*Heteronuclear Multiple Bond Correlation*
HMQC	:	*Heteronuclear Multiple Quantum Coherence*
HPLC	:	*High Performance Liquid Chromatography*
HT	:	*5-Hydroxytryptamine*
KOR	:	*Kappa Opioid Receptor*
M	:	*Muscarinic*
MOR	:	*Mu Opioid Receptors*
NET	:	*Norepinephrine Transporter*
NIMH-PDSP	:	*National Institute of Mental Health Psychoactive Drug Screening Program*
NMR	:	*Nuclear Magnetic Resonance*
rBZP	:	*Rat Benzodiazepine*
SERT	:	*Serotonin Transporter*

1. INTRODUCTION

Amentoflavone (I3'-II8 biapigenin) is a biflavonoids found mainly in the gymnosperms that has good binding to GABA subtypes receptors with affinity similar to diazepam [1-3], It exerts anxiolytic activity in mice without many of unwanted effects of benzodiazepines [4]. Amentoflavone is a common biflavonoid in famous traditionally used plants for treatment of CNS related disease, e.g. *Hypericum perforatum* L. (Hypericaceae) [1] and *Ginkgo biloba* [4]. A comprehensive analysis of several pure compounds isolated from the crude extract of *Hypericum perforatum* L.″ St. John's wort", a well-known antidepressant drug, revealed that amentoflavone significantly inhibited binding at serotonin (5-HT1D, 5-HT2C), D3-dopamine, δ-opiate, benzodiazepine receptors [1] and opioid receptors [5]. Two biflavonoids, agathisflavone and amentoflavone isolated from the leaves of *Rhus pyroides* (Anacardiaceae) showed GABAA-benzodiazepine receptor binding activity (Ki of 28 and 37 nM, respectively) [6] and this plant is traditionally used to treat delirium.

In our search for flavonoids and biflavonoids with CNS binding capabilities and consequently potential drugs for treatment of many CNS related disorders, we screened several biflavonoids and some flavonoid monomers for their CNS receptor binding. To perform that, many analogues of amentoflavone, hinokiflavone and flavonoid monomers that were previously isolated from *Cycas* leaves [7,8] were selected. Methyl derivative of some major compounds were also prepared. Both natural and semi-synthetic derivatives were subjected to *in vitro* screening of their CNS activity using the resources of the National Institute of Mental Health Psychoactive Drug Screening Program (NIMH-PDSP) at the University of North Carolina at Chapel Hill following the described protocols [9]. Several new interactions between these compounds and CNS receptors and transporters were observed. The structure elucidation of the new derivative 7,7″,4‴-trimethyldihydrohinokiflavone and complete NMR assignment of 7,7″,4‴-trimethyl-2,3-dihydroamentoflavone are presented.

2. MATERIALS AND METHODS

2.1 Chemicals

Chemical codes were given by NIMH-PDSP; the natural isolates- previously isolated and characterized from *Cycas revoluta* and *C. circinalis* [7,8]- were amentoflavone (16317), 2,3-dihydroamentoflavone (16318), tetrahydroamentoflavone (16319), 2,3-dihydrohinokiflavone (16320), naringenin (16321), 2,3-dihydrobilobetin (16322), epigallocatechin (16323), isoginkgetin (16324), 2″-glucosylvitexin (16520), vicenin-2 (16521), (+) catechin (16523) and (-) epicatechin (16524). The semisynthetic derivatives were 7,4′,7″,4‴-tetramethylamentoflavone (16515), 7,7″,4‴-trimethylamentoflavone (16516), 7,7″,4‴-trimethyl-2,3-dihydroamentoflavone (16517), 7,7″,4‴-trimethyl-2,3-dihydrohinokiflavone (16519) and sakurantin (16522).

2.2 CNS Receptors Binding

Radio ligand binding assays for receptors were conducted to obtain binding affinity (K_i) [9]. Detailed on-line protocols are available for all assays at the NIMH-PDSP web site (http://pdsp.cwru.edu) [10]. Forty-four receptors and transporters we used in the primary assay. The tested compounds showed ≥50% binding inhibition with only 22 receptors and transporter. The receptors were serotonin (5-HT_{1D}, 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}), adrenergic (Alpha2A, Alpha2C), dopamine (D_1 and D_5), muscarinic (M_3), GABA (GABAA and rBZP), opioid (MOR, DOR and KOR), histamine (H_1, H_2 and H_4) and sigma receptors (Sigma 1, Sigma 2) in addition to biogenic amine transporters (DAT, NET and SERT) using the resources of the NIMH-PDSP. Only compounds showing ≥50% binding inhibition in the primary assay were subjected to secondary binding assay. For initial screening, compounds were tested at a single concentration of 10 µmol/L, and K_i determinations using 12 concentrations of unlabeled ligand spanning four orders of magnitude were obtained on compounds that gave ≥50% inhibition at 10 µmol/L. The K_i values were calculated by non-linear curve fitting using GraphPad Prism. For all experiments reported here, K_i determinations were replicated 3 times and the mean ± SE of computer-derived estimates is reported.

2.3 Methylation of Flavonoids

Methylation with diazomethane (CH_2N_2) was done according to [11]. Amentoflavone, dihydroamentoflavone, dihydrohinokiflavone, and naringenin were converted to their methyl ether derivatives. Ten mg of flavonoids were separately dissolved in the 1 mL methanol then treated at 0° with excess ethereal CH_2N_2 over a period of 2 h. The reaction was monitored with TLC. When the reactants formed two products, the products were isolated using HPLC and their structure was elucidated using NMR techniques (^{1}H NMR, ^{13}C NMR, HMQC and HMBC).

2.4 Structure Characterization

Naringenin (16321) was methylated to produce (sakurantin, 16522).

2.4.1 Sakurantin (16522): White needles from water/methanol

^{1}H NMR (400 MHz, Acetone-d_6) δ 12.13 (s, 5-OH), 7.38 (d, *J* = 8.2, H-2′,H-6′), 6.89 (d, *J* = 8.2, H-3′,H5′), 6.025 (s, H-8), 6.033 (s, H-6), 5.46 (dd, *J* = 12.9, 2.8, H-2), 3.83 (s, 7-OCH_3), 3.19 (dd, *J* = 17.1, 12.9, H-3ax), 2.74 (dd, *J* = 17.1, 2.8, H-3eq). ^{13}C NMR (100 MHz, Acetone-d_6): δ196.7 (C-4), 167.9 (C-7), 164.1(C-5), 163.3 (C-9), 157.8 (C-4′), 129.7 (C-1′), 128.1 (C-2′,C-6′), 115.2 (C-3′,C-5′), 102.8 (C-10), 94.5 (C-6), 93.6 (C-8), 79.1 (C-2), 55.3 (7-OCH_3), 42.59 (C-3). NMR data was consistent with literature [12].

Amentoflavone was methylated into 7,4′,7″,4‴-tetramethylamentoflavone (16515) [13] and 7,7″,4‴ trimethylamentoflavone (16516) [14].

Dihydroamentoflavone (16318) was methylated into its trimethyl derivatives 7, 7″, 4‴-trimethyldihydroamentoflavone. This compound was reported once as a natural product isolated from *Podocarpus taxifolia* leaves and was named podocarpusflavanone [15] and only ^{1}H NMR data of its acetate could be traced. That is the first report of its ^{13}C NMR data.

2.4.2 7, 7″, 4‴-trimethyldihydroamentoflavone (16517): Yellow powder

^{1}H NMR (400 MHz, DMSO-d_6) δ 13.17 (s, H-5 ″), 12.11 (s, H-5), 7.67 (dd, *J* = 8.6, 3.9 Hz, H-2 ′″, 6′″), 7.46 – 7.40 (m, H-6′), 7.39 (d, *J* = 4.0 Hz, H-2′), 7.03 (d, *J* = 8.3 Hz, H-5′), 6.96 (d, *J* = 8.6 Hz, H-3′″, 5′″), 6.88 (s, H-3″), 6.62 (s, H-6″), 6.07 (d, *J* = 1.8 Hz,H-8), 6.05 (dd, *J* = 4.3, 2.3 Hz, H-6), 5.54 (d, *J* = 13.0 Hz, H-2), 3.81* (s, 4′″-OCH_3), 3.79* (d, *J* = 2.5 Hz, 7″-OCH_3), 3.74 (d, *J* = 4.1 Hz, 7-OCH_3), 3.38 (dd, *J* = 17.1, 13.1 Hz, H-3ax), 2.83 – 2.71 (m, H-3eq).

^{13}C NMR (100 MHz, DMSO-d_6) δ 197.4 (C-4), 182.9 (C-4″), 167.8 (C-7), 163.9 (C-5), 163.7(C-2″), 163.4 (C-9), 163.2 (C-5″), 162.8 (C-4‴), 161. 6 (C-7″), 156.5 (C-4′), 153.9 (C-9″), 132.1 (C-2′), 131.6 (C-1′), 128.9 (C-6′), 128.6 (C-2‴,6‴), 123.3 (C-1‴), 119.0 (C-3′), 116.0 (C-5′), 114.9(C-3‴, 5‴), 106.3 (C-8″), 104.6 (C-10″), 103.5 (C-3″), 103.1 (C-10), 95.9 (C-6″), 95.1 (C-6), 94.2 (C-8), 79.6, 79.2 (C-2), 56. 8* (7″-OCH_3), 56.3* (4‴-OCH_3), 55.9* (7-OCH_3), 42.7 (C-3).

Dihydrohinokiflavone (16320) was methylated into 7, 7‴, 4‴-trimethyl dihydrohinokiflavone (16519). This is a new derivative and its NMR data are reported for the first time. Its key HMBC correlation that aided unambiguous assignment of its ^{13}C NMR data is shown in Fig. 2.

2.4.3 7, 7″, 4‴-trimethyldihydrohinokiflavone (16519)

^{1}H NMR (400 MHz, $CDCl_3$) δ 12.88 (s, 5‴-OH), 12.03 (s, 5-OH), 7.88 (d, *J* = 8.9, H2‴,6‴), 7.38 (d, *J* = 8.7, H2′.6′), 7.05 (d, *J* = 8.9, H3‴, 5‴), 7.01 (d, *J* = 8.7, H3′.5′), 6.65 (s, H-8‴), 6.64 (s, H-3‴), 6.08 (d, *J* = 2.3, H-8), 6.05 (d, *J* = 2.3, H-6), 5.39 (dd, *J* = 13.0, 3.0, H-2), 3.93 (s, 7‴-OCH_3), 3.92 (s, 4‴-OCH_3), 3.81 (s,7-OCH_3), 3.10 (dd, *J* = 17.2, 13.0,H-3_{ax}), 2.81 (dd, *J* = 17.2, 3.0, H-3_{eq}).

^{13}C NMR (101 MHz, $CDCl_3$) δ 196.0 (C-4), 182.5 (C-4‴), 168.0 (C-7), 164.4 (C-5), 164.1 (C-2‴), 162.9 (C-9), 162.8 (C-4‴), 158.7 (C-7‴), 158.4 (C-4′), 154.5 (C-9‴), 153.6 (C-5‴), 131.8 (C-1′), 128.1(C-2‴,6‴) , 127.7 (C-2′.6′), 126.4 (C-6‴), 123.4 (C-1‴), 115.2 (C-3‴,5‴), 114.6 (C-3′,5′), 106.2 (C-10‴), 104.3 (C-3‴), 103.2 (C-10), 95.1 (C-6), 94.2 (C-8‴), 90.9 (C-8), 79.0 (C-2), 56.5* (7-OCH_3), 55.67* (4‴-OCH_3), 55.56* (7″-OCH_3), 43.2 (C-3).

3. RESULTS AND DISCUSSION

Several natural and semisynthetic flavonoids and biflavonoids were screened for primary and secondary binding with CNS receptors and biogenic amine transporters. Several new interactions were observed. Biflavonoids showed significant interactions with rat benzodiazepine receptor (rBZP), GABAA receptor, dopamine transporter (DAT) and norepinephrine transporter (NET) (Table 1). Interaction with rBZP receptor and GABAA receptors can lead to anticonvulsant drug leads [16]. Amentoflavone (16317) showed significant binding with rBZP receptor and NET (K_i= 22 nM and 50.5 nM respectively) while dihydroamentoflavone (16318) bound with rBZP receptor with K_i= 295 Nm (Fig. 1B). Tetrahydroamentoflavone (16319) bound with rBZP and GABAA receptors (K_i= 196 nM and 239 nM respectively) and finally dihydrobilobetin interacted with NET with K_i= 313.5 nM. Complete methylation of amentoflavone created 7,4′,7″,4‴-tetramethylamentoflavone (16515) which is completely inactive towards the tested receptors and transporters. Flavonoid monomers showed different interactions since significant binding with DAT was observed for (+) catechin (16523) (K_i= 1 nM) and sakurantin (16522) (K_i=1.6 Nm, Fig. 1D). Di-tri and tetramethyl ether derivatives of amentoflavone showed poor or no binding at all with the tested receptors (Tables 1, 2). Naringenin (16321) itself was inactive while its 7-methyl derivative (sakurantin) showed significant binding with DAT. (+) Catechin was active towards DAT while its 3-epimer (-) epicatechin (16524) and epigallocatechin (16323) were completely inactive which indicates a correlation between hydroxyl group position in ring B and C of flavan-3-OLs and their interaction with Dopamine transporter. Flavonoid-*C*-glycosides; 2″-glucosylvitexin (16520) and Vicenin-2 (16521) interacted with Sigma 2 receptors (K_i = 893 nM and 114 nM respectively), Sigma-2 ligands are potentially useful as cancer diagnostics, anticancer therapeutics, or adjuvant anticancer treatment agents [17].

The neurotransmitter transporters have been proven to be important targets for drug discovery in the central nervous system, particularly for antidepressants [18]. DAT is a type of protein that actively transports the neurotransmitter dopamine within nerve synapses. Some potent and selective or mixed NET inhibitors have been successfully developed to treat a variety of mental disorders such as depression and attention deficit hyperactivity disorder (ADHD) [19]. During methylation of biflavonoids with diazomethane, the 5-hydroxyl groups in the flavonoids and biflavonoids were resistant to methylation. This may be attributable to hydrogen bonding with the 4-carbonyl group. The 4′-hydroxyl group in biflavonoids was the last one to be methylated due to steric hindrance [4]. We were obliged to use two-digit in recording the chemical shift of very close signals in ^{13}C NMR assignments and values showing common superscripts may be interchanged.

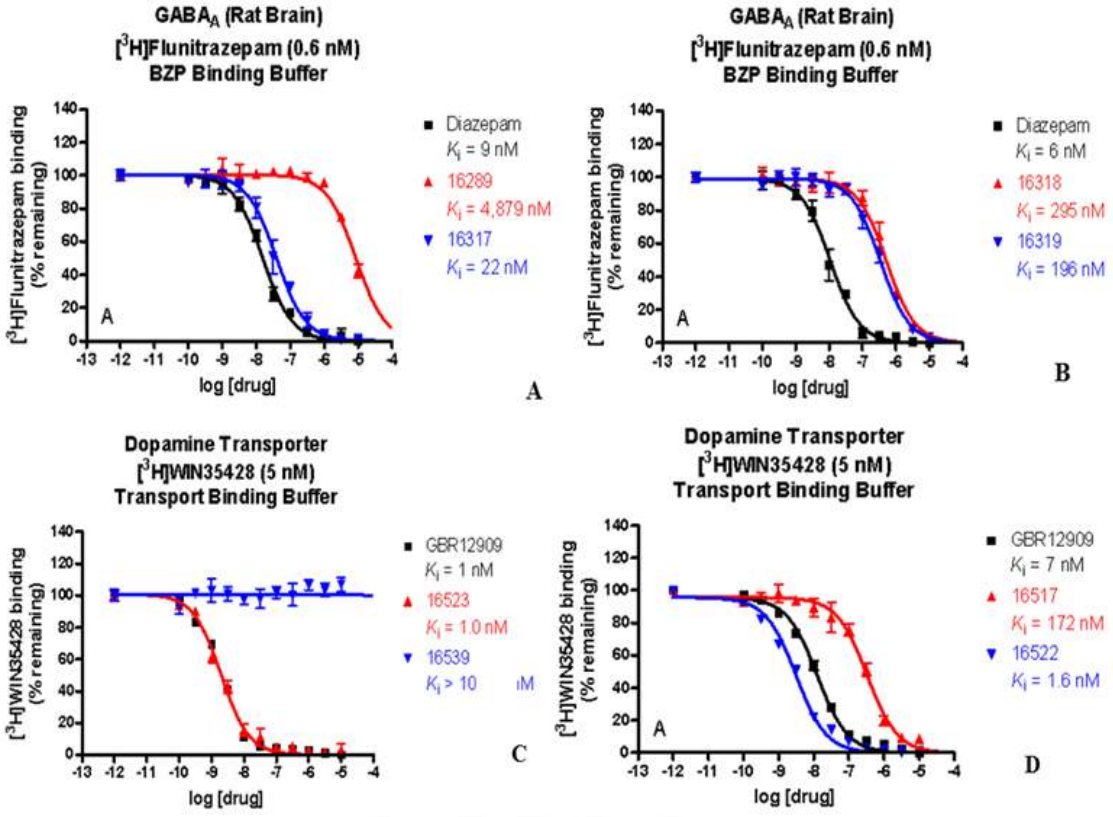

Fig. 1. Significant secondary binding results (Ki values) of flavonoids towards CNS receptors and transporters. Amentoflavone (16317); A, 2, 3-dihydroamentoflavone (16318) tetrahydroamentoflavone (16319) to Rat BZP receptors; B, and catechin (16523); C, sakurantin (16522); D, to Dopamine transporter. Data represents positive control (black line) and two PDSP compounds

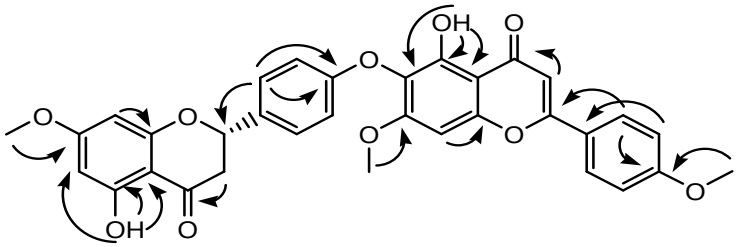

Fig. 2. Key HMBC correlations of 7,7″,4‴-trimethyl dihydrohinokiflavone (16519)

3.1 Structure–activity Relationship

Analyzing the CNS binding results, we found out that methylation of biflavonoids decreased their ability to bind with CNS receptors and transporters except DAT (i.e. amentoflavone was very active and its tetramethyl derivative was completely inactive). Concerning interaction with DAT, 7,7″,4‴-trimethyl-2,3-dihydroamentoflavone was the most active among biflavonoids. Methylation of naringenin created new binding capability of sakurantin. In flavan-3-ols, β-OH at C-3 in (+) catechin and ortho-dihydroxy groups at ring B are essential for activity towards DAT since (-) epicatechin which has α-OH at C3 and epigallocatechin with an extra hydroxyl group in ring B are completely inactive.

Table 1. Significant primary and secondary binding (Ki values) results of some natural flavonoids and biflavonoids with CNS receptors and transporters

Receptor	16317	16318	16319	16320	16321	16322	16323	16324	16520	16521	16523
5-HT1D		(58.6) 1894±180									
5-HT2A		(51.8) 4019±357				(81.5) 5306±545					
5-HT2B		(64.2) 1910±225									
5-HT2C	(51.6) 8765±734	(74.8) 1828±182	(50.4) 9890±1348	(62.4) 2568±311							
Alpha2A		(71) >10,000									
Alpha2C	(61.5) 1231±101.3										
BZP	(99.3) 22±0.8	(97.9) 295±12	(97.5) 196±6	(96.8) 1389±56		(56.7) 5218±291					
D1		(66) 3792±312									
D5	(101.6) >10,000	(100.9) >10,000	(88.3) >10,000	(70.1) >10,000							
DAT	(82) 473±34	(77.2) 308±15	(86.7) 245±15	(88) >10,000	(90) >10,000	(84.8) >10,000	(79) >10,000				(93) 1±0.05
DOR	(78) 452±54	(95.9) 538±54	(70.2) 2054±166					(54.3) 1435±128			
GABAA	(71.9) >10000		(62.6) 239±22								
H1	95.5) >10,000	(91.2) >10,000	(89.6) >10,000	(88.6) >10,000	(67.5) >10,000	(97.8) >10,000	(102.9) >10,000	(103) >10,000			
H2		(68.6) 1772±197									
H4		(72.2) 3573±596.7									
KOR		(70.8) 2273±239	(50.8) 3072±294								
M3		(54.6) 7854±870	(56.1) >10,000			(56) >10,000	(50.4) >10,000	(55) >10,000			

Receptor	16317	16318	16319	16320	16321	16322	16323	16324	16520	16521	16523
MOR		(71.3) 1682 ±92									
NET	(59.2) 50.5 ±5	(83.3) 10,000	(80.8) 10,000	(76.9) 10,000	(73.7) 10000	(80.2) 313.5 ±24	(64.6) 7,480 ±502				
SERT						(75.5) >10,000	(62.4) >10,000				
Sigma 1		(65.9) 4790 ±532				(59.7) 5383 ±560				(87.9) 1812 ±92	
Sigma 2		(100.3) 1679 ±208				(97) 1924 ±179			(59.6) 893 ±56	(95.3) 114 ±7	

Primary binding result between brackets (% inhibition of radio ligand binding), Ki value (nM)±SE underneath. Amentoflavone (16317), 2,3-dihydroamentoflavone (16318), tetrahydroamentoflavone (16319), 2,3-dihydrohinokiflavone (16320), naringenin (16321), dihydrobilobetin (16322), epigallocatechin (16323), isoginkgetin (16324), 2"-glucosylvitexin (16520), vicenin-2 (16521), catechin (16523) The CNS receptors were serotonin (5-HT1D, 5-HT2A, 5-HT2B and 5-HT2C), adrenergic (Alpha2A, Alpha2C), dopamine (D1 and D5), muscarinic (M3), GABA (GABAA and rBZP), opioid (MOR, DOR and KOR), histamine (H1, H2 and H4) and Sigma receptors (Sigma 1, Sigma 2) in addition to biogenic amine transporters (DAT, NET and SERT) using the resources of the NIMH-PDSP

Table 2. Significant primary and secondary binding (Ki values) results of some semisynthetic flavonoids and biflavonoids with CNS receptors and transporters

Receptor	16516	16517	16518	16519	16522
5-HT1D					(58) 10,000
5-HT2B			(58.9) 7,352.5 ±463		(60.8) 4,304±209
5-HT2C			(50.5) >10,000	(53.2) >10,000	
BZP			(67.2) 1472 ±127.2		
DAT	(54.3) >10,000	(55) 172±8.9			(60.9) 1.6 ±0.099
DOR			(56.8) 2840 ±95		
H2			(60.2) 4279 ±228.2		
KOR			(63) 3530 ±126	(75.1) 6837±472	
SERT	(52.7) >10,000		(52.3) >10,000		

Primary binding result between brackets (% inhibition of radio ligand binding), Ki value (nM)±SE. 7,4′,7″,4‴-tetramethylamentoflavone (16515), 7,7″,4‴-trimethylamentoflavone (16516), 7, 7″, 4‴-trimethyldihydroamentoflavone (16517), 7-methyldihydroamentoflavone (16518), 7, 7″, 4‴-trimethyl dihydrohinokiflavone (16519), sakurantin (16522), The CNS receptors were serotonin (5-HT1D, 5-HT2B and 5-HT2C), GABA (rBZP), opioid (DOR, KOR), histamine (H2) in addition to biogenic amine transporters (DAT and SERT) using the resources of the NIMH-PDSP

4. CONCLUSION

The major finding of this study is those distinct chemical constituents of flavonoids and biflavonoids which showed several new interactions with CNS receptors and biogenic amine transporters. The data clearly demonstrate that some of the investigated substances showed an unanticipated binding inhibition in several receptor assays. The most potent binding activities were observed for the biflavonoids amentoflavone, dihydroamentoflavone to rBZP, GABAA receptor and NET. 7,7″,4‴-trimethyl-2,3-dihydroamentoflavone and the flavonoid monomers (+) catechin and sakurantin to DAT. Methylation of biflavonoids negatively affected the binding ability while methylation of naringenin positively affected the results. (+) catechin was very active while (-) epicatechin and epigallocatechin were completely inactive towards DAT. Our study points out some potential antidepressant, anticonvulsant natural drug leads.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Butterweck V, Nahrstedt A, Evans J, Hufeisen S, Rauser L, Savage J, Popadak, et al. *In vitro* receptor screening of pure constituents of St. John's wort reveals novel interactions with a number of GPCRs. Psychopharmacol (Berl). 2002; 162(2):193–202.
2. Svenningsen AB, Madsen KD, Liljefors T, Stafford GI, van Staden J, Jäger AK. Biflavones from *Rhus* species with affinity for the GABA(A)/benzodiazepine receptor. J Ethnopharmacol. 2006;103(2):276–80.
3. Nielsen M, Frøkjaer S, Braestrup C. High affinity of the naturally-occurring biflavonoid, amentoflavon, to brain benzodiazepine receptors *in vitro*. Biochem Pharmacol. 1988;37(17):3285–7.
4. Hanrahan JR, Chebib M, Davucheron NLM, Hall BJ, Johnston GAR. Semisynthetic preparation of amentoflavone: A negative modulator at GABA A receptors. Bioorg Med Chem Lett. 2003;13:2281–4.
5. Katavic PL, Lamb K, Navarro H, Prisinzano TE. Flavonoids as opioid receptor ligands: Identification and preliminary structure-activity relationships. J Nat Prod. 2007;70(8):1278–82.

6. Stafford GI, Pedersen ME, Van Staden J, Jäger AK. Review on plants with CNS-effects used in traditional South African medicine against mental diseases. J Ethnopharmacol. 2008;119(3):513–37.
7. Moawad A, Hetta M, Zjawiony JK, Ferreira D, Hifnawy M. Two new dihydroamento-flavone glycosides from *Cycas revoluta*. Nat Prod Res. 2014;28(1): 41–7.
8. Moawad A, Hetta M, Zjawiony JK, Jacob MR, Hifnawy M, Marais JPJ, Ferreira D. Phytochemical investigation of *Cycas circinalis* and *Cycas revoluta* leaflets: Moderately active antibacterial biflavo-noids. Planta Med. 2010;76(8):796–802.
9. Besnard J, Ruda GF, Setola V, Abecassis K, Rodriguiz RM, Huang XP, et al. Automated design of ligands to poly-pharmacological profiles. Nature. 2012; 492(7428):215–20.
10. Available:https://pdspdb.unc.edu/pdspWeb/content/PDSP%20Protocols%20II%20201 3-03-28.pdf, visited April 4, 2017.
11. Markham KR. The structures of amentoflavone glycosides isolated from *Psilotum nudum.* Phytochemistry. 1984; 23(9):2053–6.
12. Vasconcelos JMJ, Silva AMS, Cavaleiro JAS. Chromones and flavanones from *Artemisia campestris* subsp. *maritima*. Phytochemistry. 1998;49(5):1421–4.
13. Markham KR, Sheppard C, Geiger H. ^{13}C NMR studies of some naturally occurring amentoflavone and hinokiflavone biflavonoids. Phytochemistry. 1987; 26(12):3335–7.
14. Carbonezi CA, Hamerski L, Gunatilaka AAL. Bioactive flavone dimers from *Ouratea multiflora* (Ochnaceae). Brazilian J Pharmacogn. 2007;17(3):319–24.
15. Roy SK, Qasim MA, Kamil M, Ilyas M. Biflavones from the genus *Podocarpus*. Phytochemistry. 1987;26(7):1985–7.
16. Sieghart W. GABA(A) benzodiazepine receptors and epilepsy. Wien Klin Wochenschr. 1990;102(7):197–201.
17. Huang YS, Lu HL, Zhang LJ, Wu Z. Sigma-2 receptor ligands and their perspectives in cancer diagnosis and therapy. Med Res Rev. 2014;34(3):532–66.
18. Amara SG, Kuhar MJ. Neurotransmitter transporters: Recent progress. Annu Rev Neurosci. 1993;16:73–93.
19. Zhou J. Norepinephrine transporter inhibitors and their therapeutic potential. Drugs Future. 2004;29(12):1235–44.

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