Scholars Academic Journal of Biosciences (SAJB)
Abbreviated Key Title: Sch. Acad. J. Biosci.
ISSN 2347-9515 (Print)
ISSN 2321-6883 (Online)

A Unit of Scholars Academic and Scientific Society, India
www.saspublishers.com

Pharmacology

Molecular Simulation of Flavonoids as Inhibitors for Influenza A Virus Hemagglutinin and Neuraminidase Receptors of Known Strains

Hasan Mahmood Abo Almaali*, Haifaa Rasheed Muhsin, Waleed Shoky Abass Alabrahemy, Mustafa Yousif Rasheed Alrubaie, Ali Mudhafar Jahil Almhanna
College of Pharmacy, Kerbala University, Kerbala, Iraq

Original Research Article

***Corresponding author**
Hasan Mahmood Abo Almaali

Article History
Received: 29.12.2017
Accepted: 16.01.2018
Published:30.01.2018

DOI:
10.36347/sajb.2018.v06i01.005

Abstract: The influenza virus is known to cause illnesses worldwide, sometimes epidemics or even cause pandemics. Many efforts are done to reach the best treatment strategy to reach agents able to cure this disease without affecting by its continuous evolution patterns. So that, in current study a 56 flavonoid natural compounds were investigated upon their potential to be inhibitors to influenza receptors, hemagglutinin and neuraminidase. Consequently, this done using molecular docking to certain active sites on these receptors. Additionally, several drug likeliness properties were calculated for flavonoids with best docking score. Moreover, the present study resulted with two molecules, the first (flavonol 3-O-rutinoside) with high docking score to attachment to 12 out of 18 hemagglutinin from known strains, and the remaining strains were compensated by the second one (theaflavin). Additionally, the same results were founded for neuraminidase, as (gallate gallo-catechin) with high docking score attachment to 9 out of 11known strains, and the remaining strains compensated by (flavonol 3-O-d-glucoside). In conclusion, all of (flavonol-3-O-rutinoside) and (theaflavin) may have the potential to work as hemagglutinin inhibitors, and (gallate gallo-catechin) with (Flavonol-3-O-d-glucoside) may have the potential to be neuraminidase inhibitors. Accordingly, the usage of all of them in the same drug formula may cover all known strains of both of hemagglutinin and neuraminidase, preventing this virus from establishing infection and spreading for all of humans and animals hosts. These results may provide wet laboratory experiments with data to base on it in anti-influenza agents development.
Keywords: Influenza, hemagglutinin, neuraminidase, flavonoids, strains.

INTRODUCTION

The season which mostly influenza infections raised and spread is usually the winter, usually from December to March. The infection spread from person to person may happen within approximately two-meters distance, and its spreading may happen before symptoms appeared in patients [1]. So that everyone is subjected at certain times to influenza infection.

Influenza A virus classified according to their two proteins, the hemagglutinin, and neuraminidase. Additionally, 18 types of hemagglutinin with 11 neuraminidase were discovered yet. The combinations of these types will specify host targets [2]. The H1N1 and H3N3 were causes human influenza, remaining types causes certain animals influenza, and some of the influenza A types infect both humans and certain animals [3].

Additionally, one of the obstacles against influenza infection treatment is its continuous changing through antigenic drift and shift, which confer it's continues resistance to therapies, both vaccines, and other treatments agents [4].

In addition to vaccines, there are two anti-influenza drug targets avalabe, the nuraminidase inhibitors (like oseltamivir) and the M2 protein blockers (like amantadine) [5].

From all of mentioned above, there is growing need to investigate more agents with a new strategy covering known strains and this may confer potential treatment for newer strains.

MATERIALS AND METHODS

Materials

Influenza virus receptors

The three dimensional structure for both neuraminidase (strains from 1 to 11) and hemagglutinin (strains from 1 to 18) were derived from Protein Data Bank with PDB IDs shown in tables 1 and 2 (H8, H11, and H12 were not available). The center of Neuraminidase active site was R118, E119, and I222, in addition the center of selected hemagglutinin active site were Y98, W153, H183, and L194.

Table-1: Neuraminidase types with corresponding PDB IDs used in current study

Neuraminidase type	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11
PDB ID	2hu4	1ivf	4hzv	2htw	3sal	1v0z	4qn7	2htu	1f8b	4gdi	4mc7

Table-2: Hemagglutinin types with corresponding PDB IDs used in current study

Hemagglutinin type	H 1	H2	H3	H4	H5	H6	H7	H9	H10	H13	H1 4	H15	H16	H17	H18
PDB ID	4e db	2wr d	3zt j	5xl 2	5e2 y	5t0 b	4lk h	1js h	4xq 5	4kp q	3ey j	5tg 8	4f2 3	4i7 8	4k3 x

Flavonoids compounds

A 56 flavonoid compounds were used in current study for molecular simulation purposes against both neuraminidase and hemagglutinin receptors. These included chemical compounds listed in table 1, all of them were derived from PubChem database [6].

Table-3: Flavonoids compounds

Flavan3ol	Flavanones	Isoflavones	Anthocyanin	Flavones	Flavonols
Fisetinidol	Naringenin	glycitein	Anthocyanin-3-o-beta-d-glucoside	Mitoflaxone	Narcissoside
Gallo-catechin gallate	Poncirin	3-o-methylorobol	Pelargonidin	3-Hydroxyflavone	Flavonol-3-O-rutinoside
Catechin gallate	Isosakuranin	Biochanin A	Flavylium	3-Methyl-6-piperidinomethyl-flavone hydrochloride	Flavonol 3-O-D-glucoside
Gallo-catechin	Pinocembrin	Formononetin	Delphinidin	7-(diethoxyphosphinyloxy) flavone	3-O-d-galactoside
Guibourtinidol	Eriodictyol	Pemerlast	Peonidin-3-glucoside	7-Hydroxyflavone	Glycyrrhiza
Catechin	Hespertin	Pallidiflorin	Rosinidin	Chrysin	3-hydroxyflavone
Mesquitol	Butin	Genistein	Malvidin	FLAVONE	Retusin
Theaflavins	Sterubin	Odoratin	Petunidin	Quercetin	Santin
Afzelechin	Homoeriodictyol	Irisolidone	Cyanidin-3-o-rutinoside	REC 7-0112	-
Proanthocyanidins	Hesperidine	Daidzein	-	-	-

Methods

Programs Software and Databases

Several databases and software tools both online and standalone were used, includeing the following: 1-click docking [7], UCSF chimera [8], Protein data bank (PDB) [9].

Strategy of potential inhibitors selection

All of 56 studied flavonoids including flavanones, flavan-3-ol, flavones, anthocyanin, isoflavones, and flavonols flavonoids groups were subjected to molecular docking through MCULE server using 1-click docking tool against both hemagglutinin and neuraminidase active sites. The docking score smaller than -7 was considered a stable attachment of flavonoid to hemagglutinin or neuraminidase active sites. Additionally, the compounds cover larger number of strains considered wide spectrum inhibitors to influenza drug targets which differs among strains.

RESULTS

Table-4: molecular docking of studied flavonoids to hemagglutinin strains from 1 to 18, with score stronger than-7

Flavonoids	H1	H2	H3	H4	H5	H6	H7	H9	H10	H13	H14	H15	H16	H17	H18	No. of positive
Flavonol **3-O-rutinoside**	+	+	+	+		+	+		+		+	+	+	+	+	**12**
Theaflavin	+	+	+	+	+			+	+	+	+	+			+	**11**
Catechin gallate		+		+	+	+				+	+	+	+			8
Anthocyanin-3-o-beta-d- glucoside	+			+	+		+		+		+	+	+			8
Cyanidin-3-o-rutinoside		+	+			+		+	+		+				+	7
gallo-catechin gallate	+	+				+			+			+			+	6
Poncirin		+	+	+						+	+	+				6
Hesperidin	+	+	+	+	+						+					6
Glycyrrhiza	+	+		+	+			+				+				6
Proanthocyanidins	+		+							+	+	+				5
narcissoside	+			+							+	+			+	5
Fisetinidol	+	+										+				3
Delphinidin					+							+	+			3
Gallo-Catechin	+											+				2
3-Methyl-6-piperidinomethyl-flavone hydrochloride					+	+										2
Eriodictyol					+							+				2
Hespertin					+							+				2
3-O-D-galactoside						+				+						2
Guibourtinidol												+				1
catechin												+				1
Afzelechin												+				1
Mitoflaxone												+				1
Chrysin												+				1
7-Hydroxyflavone												+				1
Quercetin												+				1
Pelargonidin												+				1
Peunidin-3-glucoside												+				1
malvidin												+				1
Butin												+				1
Isosakuranin					+											1
Naringenin												+				1
Pinocembrin												+				1
Homoeriodictyol												+				1
Formononetin		+														1
Flavonol 3-O-D-glucoside												+				1

Table-5: molecular docking of studied flavonoids to 11 neuraminidase strains with score stronger than -7

Flavonoid	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11	No. of positive
Gallo-catechin gallate	+	+	+		+	+	+		+	+	+	**9**
Fisetinidol	+	+			+	+	+		+	+	+	8
Catechin gallate	+	+			+	+	+		+	+	+	8
Poncirin		+		+		+	+		+	+	+	7
Mitoflaxone	+	+				+	+		+	+		6
3-hydroxyflavone	+	+		+		+	+		+			6
Theaflavins	+					+	+	+		+	+	6
Anthocyanin-3-o-beta-D-glucoside	+					+	+		+	+	+	6
Gallo-catechin	+	+				+	+		+			5
Catechin	+	+				+	+		+			5
Mesquitol	+	+	+			+			+			5
Quercetin		+				+	+		+	+		5
Flavonol 3-0-d-glucoside				+	+		+	+			+	**5**
Guibourtinidol	+	+				+			+			4
Chrysin		+				+	+		+			4
Flavone		+				+	+		+			4
Hesperidin						+	+		+	+		4
Narcissosid		+					+	+			+	4
Cyanidin-3-O-rutinoside						+	+			+	+	4
Pelargonidin	+					+			+			3
Homoeriodictyol		+				+	+					3
7-hydroxyflavone		+				+			+			3
Butin				+				+	+			3
3-O-rutinoside					+		+		+			3
3-O-d-galactoside					+		+		+			3
3-Methyl-6-piperidinomethyl-flavone hydrochloride						+	+			+		3
Flavylium						+	+		+			3
Delphinidin						+	+		+			3
Peonidin-3-glucoside						+	+		+			3
Petunidin						+	+		+			3
Glycyrrhiza							+		+	+		3
Afzelechin		+				+						2
Proanthocyanidins						+				+		2
Eriodictyol						+	+					2
Glycitein						+			+			2
3-O-methylorobol							+		+			2
Genistein							+		+			2
Biochanin A							+		+			2
Pinocembrin								+	+			2
Sterubin								+	+			2
Irisolidone									+	+		2
Odoratin									+	+		2
7-(Diethoxyphosphinyloxy)flavone		+										1
Hespertin						+						1
Diethyl Flavone-5,7-dioxyacetate						+						1
Rec 7-0112						+						1
Isosakuranetin								+				1
Rosinidin									+			1
Daidzein									+			1
Formononetin									+			1
Pallidiflorin									+			1
Pemirolast									+			1
O-rutinoside											+	1

Table-6: Lipinski rule of five properties of flavonoids with best docking score to hemagglutinin and neuraminidase

Property	Hemagglutinin		Neuraminidase		Normal values
	Theaflavin	Flavonol 3-O-rutinoside	Gallo-catechin gallate	3-O-d-glucoside	
Mass	564.4916	576.5873	458.3702	400.3773	Less than 500
Log P	2.2134	0.5167	2.2332	0.6387	Less than 5
H-bond acceptors	12	12	11	8	Less than 10
H-bond donors	9	6	8	4	Less than 5
Rotatable bonds	2	6	4	4	Less 10
RO5 violation	3	3	2	0	0
RO3 violations	4	3	5	5	0

Table-7: structures and docking poses for flavonoids with wide spectrum against hemagglutinin attachment

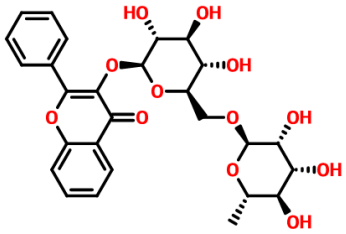
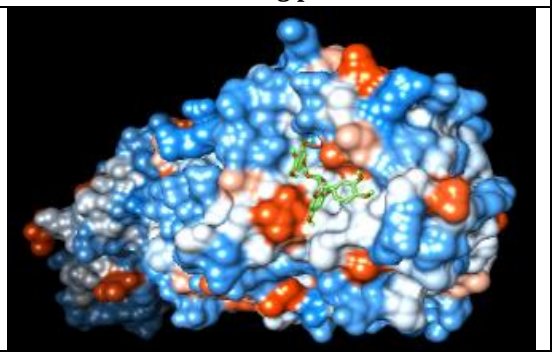
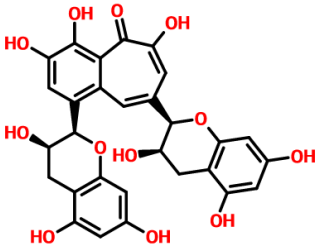
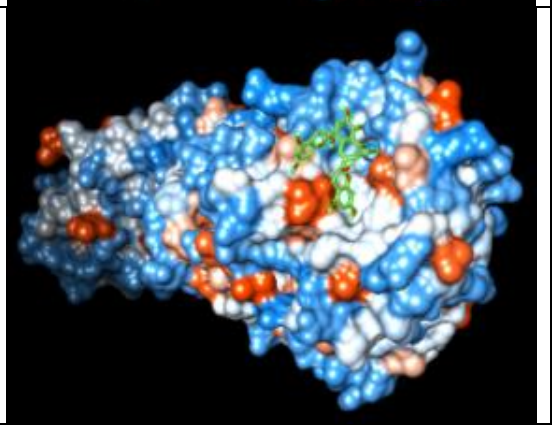
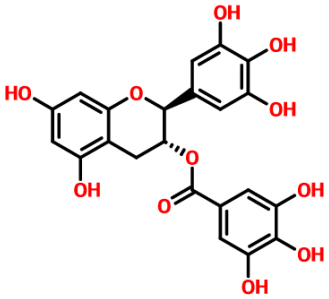
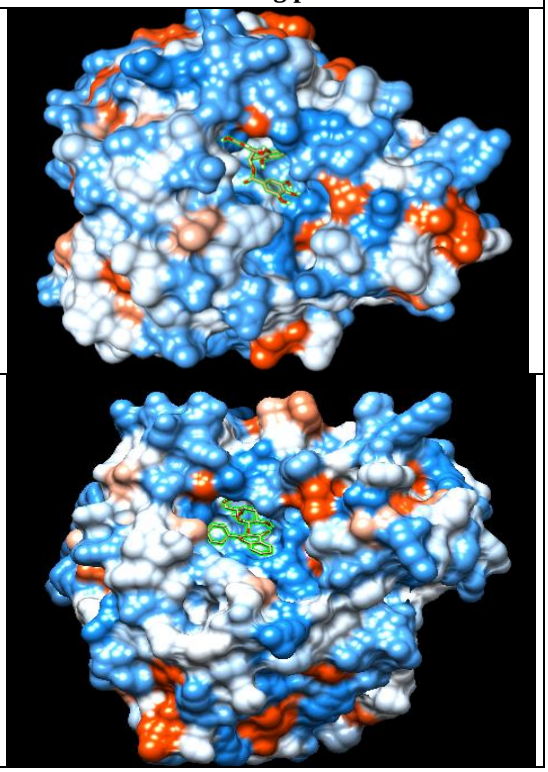
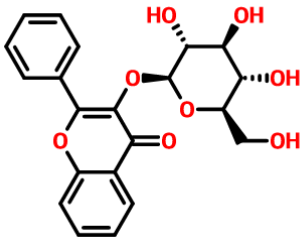
Flavonoid	Structure	Docking pose
Flavonol-3-O-rutinoside		
Theaflavin (Flavan-3-ol group)		

Table-8: structures and docking poses for flavonoids with wide spectrum against neuraminidase attachment

Flavonoid	Structure	Docking pose
Gallo-catechin gallate (Flavan-3-ol group)		
Flavonol 3-O-d-glucoside (Flavonol group)		

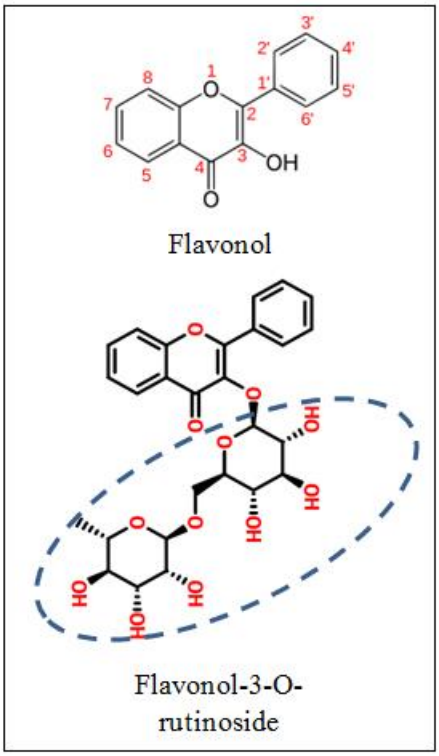


Fig-1: Rutin group configuration on flavonol

DISCUSSION

The infection caused by influenza virus around the world resulting to seasonal infections or sometimes pandemics, and their continuous changing of its genomes and proteomes mad a growing treatment obstacles to both vaccinations and small molecules drugs [10]. Moreover, in addition to vaccines, several agents, and drugs used in the treatment of influenza infection used previously, these are grouped basing on their drug targets in or on influenza particles, in

addition to viral entry to hosts cells blocker class. Usually, the main drug targets for influenza viruses were hemagglutinin, neuraminidase, M2 protein, polymerase, nucleoprotein [11]; others may be discovered in the future.

On the other hand, the natural compound large group, the flavonoids were known to possess a broad range of health-enhancing effects. These included anti-oxidant, anti-inflammatory, antimicrobial, and other properties, these effects made researcher investigate the potential of particular flavonoids compounds to treat certain diseases like infections [12].

In the present study, flavonoids including subgroups of flavanones, flavan-3-ol, flavones, anthocyanin, isoflavones, and flavonols compounds were simulated to their attachment to two of influenza drug targets, the hemagglutinin and neuraminidase, to find best of them to be a candidate inhibitor for a broader range of viral strains making influenza treatment more useful and withstand drug target alterations to escape from treating agents, this may be still suitable to treat future generated strains, because of its chemical properties still confer acceptable attachment to drug targets.

The current study results showed that the best agents cover the active sites of most strains of hemagglutinin, and neuraminidase were belonged to flavan-3-ol group (including theaflavins and gallo-catechin gallate), and flavonol group of flavonoids (including flavonol 3-O-rutinoside and flavonol 3-O-d-glucoside). These agents have possessed nearly acceptable values of Lipinski rule of five properties, and deviations were not too far. And resources concerning their activity in vitro and in vivo are limited.

Several previous efforts were done to find flavonoids capable to inhibit influenza virus receptors; these included successful use of one of the flavone members, the isoscutellarein, which inhibit influenza virus neuraminidase [13, 14]. In addition, in other study done in vivo showed acceptable therapeutic prosperity of 6-hydroxyluteolin-7-O-beta-d-glucoside, nepitrin, and homoplantaginin extracted from salvia plebeia against H1N1 neuraminidase [15]. Another researchers group suggested flavone members as potent neuraminidase inhibitors to H1N1 influenza [16], all of the mentioned articles focused on certain strains of influenza (causing humans infections), and resulted with specific compounds, and their reaction with other strain is still undiscovered.

Additionally, it's demonstrated previously that rutin group has the potential to be antiviral activity toward herpes simplex virus-1 through the compounds, quercetin-3-O-rutinoside and kaempferol-3-O-rutinoside in comparison to corresponding nonglycosylated compounds quercetin, kaempfero [17]. While, in the current study, the flavonol 3-O-rutinoside also has rutin group with strong affinity to the receptors of 12 out of 15 strains of hemagglutinin, this property suggests this compound to further wet lab investigations toward influenza virus treatment.

Theaflavin, which generated from two flavan-3-ol compounds fusion, found in black tea leaves, poses suppression effect on HIV replication, reduces cholesterol, and anti-influenza hemagglutinin and neuraminidase receptors of influenza B (B/Jiangsu/10/2003), H1N1 (A/PR/8/34), and H3N2 (A/Sydney/5/97) [18], other study demonstrated strong activity to inhibit H5N1 hemagglutinin [19], additionally, in current study theaflavin subjected in simulation to Hemagglutinin strains from 1 to 18 (H8, H11, and H12 were not available) and it was with strong affinity to 11 out of tested 15 strains, including (H1, H2, H3, H4, H5, H9, H10, H13, H14, H15, and H18). This strengthens information concerning theaflavin usage in influenza treatment because it may tend to inhibit most known hemagglutinin strains (a multi-target activity), including human strains.

On the other hand, several gallocatechins were used successfully for influenza treatment as anti-avian influenza combination of "theaflavin, theaflavin-3,3′-digal (TF-3), theaflavin-3-monogallate (TF-2), theaflavin-3 gallate, theaflavin-3′-gallate, thearubigin, gallic acid and tannic acid (-)-epigallocatechin gallate (EGCG), (-)-epigallocatechin (EGC), (-) -epicatechin gallate (ECG), (+) -epicatechin (EC), (-) -gallocatechin gallate (GCG), and catechin" [20]. Another article supported these data was [21], which focused only on H5N1 influenza virus strain (A/Vietnam/1203/04) neuraminidase. While in the current study the gallo-catechin gallate has the ability to inhibit 9 out of 11 neuraminidase strains, including both humans and animal influenza viruses.

Limited data available concerning the flavonol-3-O-d-glucoside, and butin, but it compensates the weak affinity of gallo-catechin gallate attachment to neuraminidase strains N4, and N8.

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