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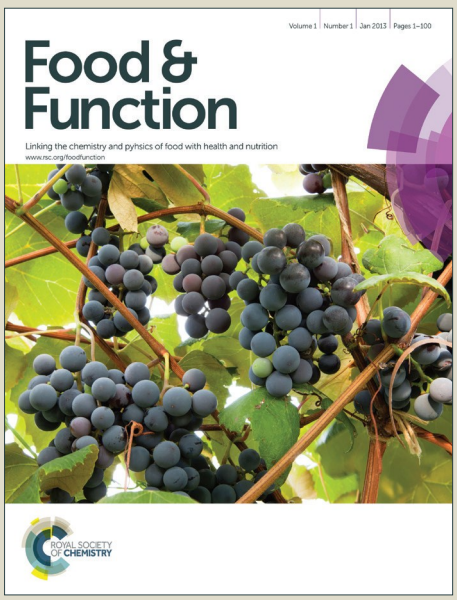

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ARTICLE

Neuraminidase inhibition of dietary chlorogenic acids and derivatives – Potential antivirals from dietary sources

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Plants rich in chlorogenic acids (CGAs), caffeic acids and their derivatives have been found to exert antiviral effects against influenza virus. In this study several dietary naturally occurring chlorogenic acids, phenolic acids and derivatives were screened for their inhibitory activity against Neuroaminidases (NAs) from *C. perfringens*, H5N1 and recombinant H5N1 (N-His)-Tag using a fluorometric assay. There was no significant difference in inhibition between the different NA enzymes. The enzyme inhibition results indicated that chlorogenic acids and selected derivatives, exhibited high activities against NAs. It seems that the catechol group from caffeic acid was important for the activity. Dietary CGA therefore show promise as potential antiviral agents. However, caffeoyl quinic acids show low bioaivailibility and are intensly metabolized by the gut micro flora, only low nM concentratrions are observed in plasma and urine, therefore a systemic antiviral effect of these compounds is unlikely. Nevertheless, gut floral metabolites with a catechol moiety or structurally related dietary phenolics with a catechol moiety might serve as interesting compounds for future investigations.

1. Introduction

Neuraminidase (NA) is one of the two glycoproteins on the surface of influenza virus, which cleaves the neuraminic acid residues to facilitate the movement of the progeny virus to affect other cells within the respiratory tract.[1] Neuraminidase inhibitors (NAIs) are the most commonly used antiviral drugs for influenza treatment. Hence, since 2010, the NAIs are the only class of antivirals recommended by the WHO for the treatment and prevention of influenza A and B viruses [2] rather than older generation adamantine-based antiviral drugs.

Currently, the most effective NAIs against both influenza A and B viruses are oseltamivir (Tamiflu) and zanamivir (Relenza), which are licensed worldwide for therapeutic and prophylactic uses.[3] However, many influenza virus mutant strains develop resistance to Tamiflu, such as the seasonal H1N1 viruses [4] and avian H5N1 strains.[5] While Relenza has a very high antiviral effect, its bioavailability is low and it is excreted rapidly by the kidneys.[6] Other NAIs such as Laninamivir and Peramivir are still in phase III clinical trials.[7]

Accordingly, new antiviral agents with greater effectiveness and better tolerability than the existing drugs are urgently required for treatment of influenza virus infection based on the neuraminidase (NA) active site. However, several polyphenols have been reported to exert antiviral effects against the influenza virus.[8-11] Moreover, 80% of registered antiviral drugs including Tamiflu are derived from natural products.[12, 13]

Chlorogenic acids (CGAs), which we define as all hydroxycinnamate esters of quinic acid,[14] are structurally remarkably similar to commercial antiviral drugs such as Ostelmavir, with viral Neuroaminidase as their validated target. Both compound classes are trisubstituted cyclohexane carboxylic acid derivatives with lipophilic moieties and a hydrogen bond acceptor in the side chain. CGAs are ubiquitous in the plant kingdom and are found in a variety of dietary sources. The estimated minimum daily intake of CGAs in the average human diet is around 1g per day. Amounts of up to 2 g per day are not uncommon [14]. The richest source in terms of dietary intake is coffee with an estimated average 200 mg CGA content per cup of coffee. Many additional fruits and vegetables are rich in CGAs in particular plants from the Asteraceae family including artichoke, lettuce or sunflower. Furthermore, other plants used in herbal medicine are rich in chlorogenic acids, caffeic acids and their derivatives were reported to possess antiviral activities,[15-21] including anti-influenza virus activity.[22-24] However, in our research, we studied for the first time the neuraminidase inhibitory

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activities of particular aceylated quinic acid and lactones derivatives on selected viral neuraminidase strains.

Hence we put forward the hypothesis that selected CGAs might act as potent Neuroaminidase inhibitors and therefore as potential antivirals.

In this contribution we therefore screened two typical traditional medicinal plants used in therapy of viral infections from the *Asteraceae* family for their CGA content, to identify potential compounds for further testing with these examples being *Ambrosia. maritima* and *Sonchus oleraceus*. The plant extracts were characterized using established LC-MS methods. Selected CGAs of these plants that also occur regularly in the human diet were tested in a fluorimetric assays fortheir NA inhibitory activity. Finally further CGA derivatives typically observed in the diet were included in the NA screen including for example chlorogenic acid lactones formed during the roasting of coffee.

2. Materials and methods

2.1 Chemicals and standards

All chemicals (analytical grade) and authentic standards of polyphenols were purchased from Sigma-Aldrich, Applichem, HWI analytic, and Phytolab (Germany). Esculin, luteolin 7-*O*-glucoside, 5-*O*-caffeoylquinic acid, 3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, 4,5-di-*O*-caffeoylquinic acid, 1,3-di-*O*-caffeoylquinic acid (cynarin), 3,4,5-tri-*O*-caffeoylquinic acid, quercetin, quercetin 3-*O*-(6-*O*-rhamnosyl-glucoside) (rutin), quercetin 3-*O*-glucoside, kaempferol 7-*O*-glucoside, kaempferol 3-*O*-(6-*O*-rhamnosyl-glucoside), luteolin, galactose, caffeic acid, shikimic acid and quinic acid were used as authentic standards.

2.2 Assay kit, drugs and neuraminidase enzymes

Amplex® Red neuraminidase (Sialidase) assay kit (A-22178) and neuraminidase from *Clostridium perfringens* were purchased from Molecular Probes Inc., Eugene, Oregon. Oseltamivir was purchased from MCE Medchem Express (Priceton, USA) and Abmole Bioscience (Hongkong, China) while zanamivir was purchased from Sigma-Aldrich (Germany), respectively. H5N1 and recombinant H5N1 (N-His)-Tag influenza virus proteins were purchased from Sino Biological Inc., Beijing, China.

2.3 Synthesis of chlorogenic acid derivatives

The synthesis of 3-*O*-caffeoylglucose (3-CG) was prepared according to a literature procedure.[25, 26] The syntheses of 1-*O*-caffeoyl-1,5-quinide (1-CQL) 9, 1-*O*-feruloyl-1,5-quinide (1-FQL) 11 and 5-*O*-dimethoxycinnamoylquinic acid (5-DQA) 15 were reported previously by our group.[27, 28] Compounds 3,4-di-*O*-feruloyl-1,5-quinide (3,4-diFQL) 10, 3,4-di-*O*-dimethoxycinnamoyl-1,5-quinide (3,4-diDQL) 12, methyl 3,4-di-*O*-caffeoylquinate (3,4-diCQM) 13, methyl 3,4-di-*O*-feruloylquinate (3,4-diFQM) 14 were synthesized according to methods reported in the literature, with some modifications.

2.4 Plant materials

A. maritima and *S. oleraceus* plant samples were freshly collected in June 2010 from their natural habitats in Khartoum North (15° 37' 44" North, 32° 37' 33" East), Khartoum, Sudan. These two plants were identified by Dr. Hayder Abdel Gadir of Herbarium of Medicinal and Aromatic Plants Research Institute (MAPRI), Khartoum, Sudan, where the specimens of *A. maritima* (No. Am-ma-02) and *S. oleraceus* (No. So-ol-01) were deposited.

2.5 Sample preparation

The samples for UPLC-Q-TOF-MS/MS and HPLC-MS^n were prepared as in our previous study.[29]

2.6 UPLC-Q-TOF-MS/MS

The UPLC system (Agilent infinity 1260 series, Germany) consisted of a binary pump, an auto sampler (G1367E), a degasser (G1322A) and a DAD detector (1315D) with a light-pipe flow cell (recording at 280 and 320 nm). This was coupled to a Ultra-High-resolution-Quadropole-Time-of-Flight (UHR-Q-TOF) (Bruker Impact HD, Bruker Daltonic GmbH, Bremen Germany) equipped with an ESI source operating on Auto-MS/MS mode. The analysis was achieved in the negative ion mode in a mass range from *m/z* 50-1200. The ESI source parameters were: capillary voltage 4.5 KV; nebulising gas pressure 1.8 Bar; drying gas temperature 200.0 °C; drying gas flow 9.0 L/min; Funnel 1RF 250.0 Vpp; transfer time 50.0 µs; and pre-pulse storage 2.0 µs. The MS data were analyzed with Data Analysis 4.2 software (Bruker Daltonics, Bremen, Germany). Internal calibration was achieved with 10 mL of 0.1 M sodium formate solution injected through a six port valve prior to each chromatographic run. Calibration was done using the High Precision Calibration (HPC).

2.7 UPLC & HPLC

The UPLC separation was achieved on a Polaris reverse phase C18 amide (RF-C18-A), 150 mm length x 2 mm inner-diameter, particle size 3 µm column (Agilent, Germany). Solvent A was water : formic acid (1000 : 0.05 v/v) and solvent B was methanol. Solvents were delivered at a total flow rate of 0.2 mL min^{-1}. The gradient profile was from 10% B to 80% B linearly in 70 min followed by 10 min isocratic and a return to 10% B at 90 min and 10min isocratic to re-equilibrate. The injection volume was 2 µl.

The HPLC separation was achieved as previously described.[30, 31]

2.8 HPLC-MS^n

HPLC-MS^n analyses were performed as shown previously [29-31].

2.9 *In vitro* neuraminidase assay

In vitro Amplex Red neuraminidase (sialidase) assay was carried out according to the manufacturer's instructions (Molecular Probes), with minor modifications. All the inhibitors were tested at three different concentrations: 1 mM, 100 µM and 10 µM. NA from *Clostridium perfringens* (0.5 U), H5N1 (60U) and recombinant H5N1 (N-His)-Tag were used to detect the compounds inhibitory activities. One unit of enzyme is

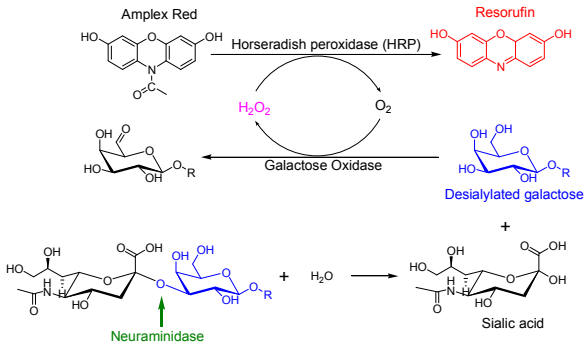

Fig. 1 The reaction scheme of neuraminidase assay using Amplex Red reagent

defined as the amount of enzyme, which catalyzing the liberation of 1 µmol of N-acetylneuraminic acid per minute at pH 5.0 and 37 °C, using bovine submaxillary mucin. The assay utilizes Amplex Red (10-acetyl-3,7-dihydroxyphenoxazine) to detect H_2O_2 generated by galactose oxidase oxidation of desialylated galactose, the end product of neuraminidase action (Fig.1). The H_2O_2, in the presence of horseradish peroxidase (HRP), reacts with Amplex Red reagent to produce the red fluorescent oxidation product resorufin (absorption and fluorescence emission maximum are approximately 563 nm and 587 nm, respectively),[32] which is detected fluorometrically using the (SAFIRE, XFLUOR4, TECAN, Austria) microplate reader. All assays were performed in triplicate, means and standard deviation values were calculated.

3. Results and discussion

3.1 Characterization of phenolic compounds in plant extracts

To substantiate our hypothesis that CGAs might act as potential antiviral agents we screened two members of the Asteraceae family of plants, a plant family particularly rich in CGAs, used in traditional medicine in the treatment of viral infections with respect to their CGA profile. *Ambrosia maritime* and *sonchus oleraceus* are extensively used as traditional herbal medicines to treat several diseases including viral infections across the African continent.[33-35] In Sudan *A. maritima* dried herb is additionally used for treatment of hypertension, diabetes, bronchial asthma, spasms, frequent urination, urinary tract infections and elimination of kidney stones.[36-38] This plant is also applied as a molluscicidal component for controlling of the intermediate hosts of faschiola and Schistosoma.[39] Moreover, two contributions have previously reported the antiviral and antifungal activities of *A. maritime*.[33, 35] On the other hand, the vegetative shoots of *S. oleraceus* have been frequently used by traditional healers to treat diabetes, diarrhoea, pneumonia and hepatitis.[34, 40] Moreover, the plant has cholagogue, laxative and emollient properties.[41] The antidiabetic, antibacterial, anti-inflammatory and antioxidant properties of *S. oleraceus* were identified in *A. maritima* and *S. oleraceus* including

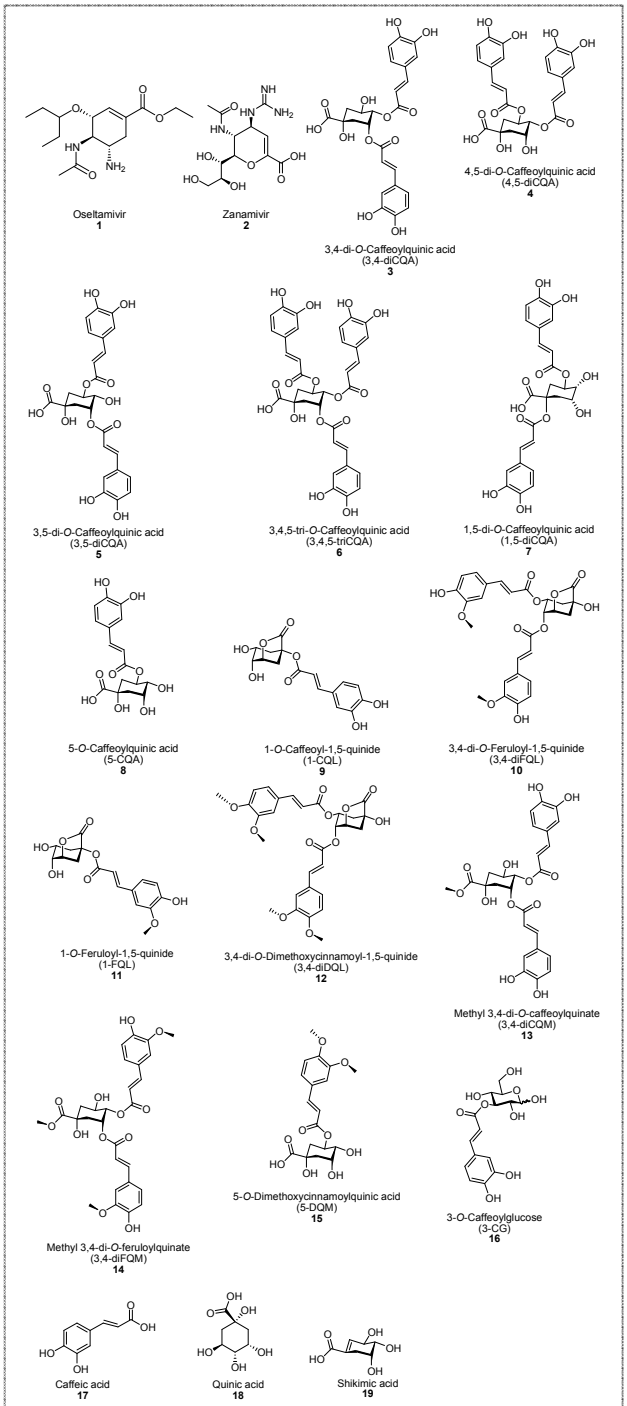

Fig. 2 Molecular structures of investigated compounds

phenols, flavonoids, proanthocyanidins, alkaloids, tannins, terpenes and steroids.[35, 42-48]

Methanolic extracts of the two antiviral medicinal plants, *A. maritima* and *S. oleraceus*, were analyzed by UPLC-ESI-Q-TOF-MS/MS and HPLC-ESI-MS^n. The first method allows assignment of molecular formulae through high resolution MS data, whereas the second approach yields additional structural information via the application of tandem MS. Comparison of

Table 1. Inhibitory activities of compounds **1**-**19** on NAs from *C. perfringens*, H5N1 and recombinant H5N1 (N-His)-Tag

No.	Compound	NA from *C. perfringens*				Influenza A neuraminidase H5N1				Recombinant influenza A neuraminidase H5N1 (N-His)-Tag			
		Absolute fluorescence		Activity relative to Tamiflu*		Absolute fluorescence		Activity relative to Tamiflu*		Absolute fluorescence		Activity relative to Tamiflu*	
		1 mM	10 µM	1 mM	10 µM	1 mM	100 µM	1 mM	100 µM	1 mM	100 µM	1 mM	100 µM
1	Tamiflu	15246	15422	1	1	14005	32476	1	1	18267	19566	1	1
2	Zanamivir	n/t	n/t	n/t	n/t	7500	13030	2	2	18941	20649	1	1
3	3,4-diCQA	231	616	66	25	3214	15649	4	2	212	802	86	24
4	4,5-diCQA	241	612	63	25	362	3763	38	8	157	678	116	28
5	3,5-diCQA	212	534	72	28	232	551	60	58	198	855	92	22
6	3,4,5-triCQA	255	739	60	20	527	3795	26	8	194	939	94	20
7	1,5-diCQA	368	662	41	23	n/t	n/t	n/t	n/t	303	1049	60	18
8	5-CQA	283	629	54	24	494	1667	28	19	193	1886	94	10
9	1-CQL	1283	2673	12	5	5838	16586	2	1	1627	14525	11	1
10	3,4-diFQL	275	2147	56	7	204	2189	68	14	194	8772	94	2
11	1-FQL	262	3140	58	4	n/t	n/t	n/t	n/t	n/t	n/t	n/t	n/t
12	3,4-diDQL	9718	7173	1	2	n/t	n/t	n/t	n/t	n/t	n/t	n/t	n/t
13	3,4-diCQM	162	395	94	38	n/t	n/t	n/t	n/t	n/t	n/t	n/t	n/t
14	3,4-diFQM	203	2639	75	5	n/t	n/t	n/t	n/t	n/t	n/t	n/t	n/t
15	5-DQA	6084	7230	2	2	n/t	n/t	n/t	n/t	n/t	n/t	n/t	n/t
16	3-CG	292	762	52	20	508	3059	27	10	309	1476	59	13
17	Caffeic acid	n/t	n/t	n/t	n/t	32	3771	442	8	15	310	1191	63
18	Quinic acid	6728	6856	2	2	1693	4675	8	6	19020	19417	1	1
19	Shikimic acid	6191	6645	2	2	n/t	n/t	n/t	n/t	n/t	n/t	n/t	n/t

* Activity relative to Tamiflu = Fl.(Tam)/Fl.(comp.); n/t = not tested

the spectral data with authentic reference standards and previously published work allowed assignment of 39 phenolic natural products. Data and assignments are given in Table S1 in the supplementary information. The main phytoconstituents were chlorogenic acids, phenolic acids, phenolic acid glycosides, flavonoid glycosides, flavonoid aglycones and coumarin derivatives. All structures and MS data used for compound assignments reported here in *A. maritima* and *S. oleraceus* are given in Table 1 with tandem MS data provided in the supplementary information. Hence within the screen candidate CGAs for testing in NA assays were identified and those CGAs as well fund in the human diet selected for further investigation.

3.2 Inhibition of influenza virus NA activities by chlorogenic acids and their derivatives

To obtain further information on the nature of the bioactive constituents traditionally an activity guided fractionation would follow. In this case we opted to follow a different and more rational approach based on the following plausible arguments. Chlorogenic acids are structurally remarkably similar to commercial antiviral drugs such as Ostelmavir, with viral Neuroaminidase as their validated target. Both compound classes are substituted cyclohexane carboxylic acid derivatives. Furthermore, other plants rich in chlorogenic acids, caffeic acids and their derivatives were reported to possess antiviral activities.[15-21] Chlorogenic acids have as well been reported to possess anti-influenza virus activity.[22-24]

We hence assumed that due to their structural similarity, the antiviral activity of *A. maritima* and *S. oleraceus* could be based on the inhibition of NA by chlorogenic acids and derivatives, the main phytochemical constituents identified in these plants. For that reason, the NA inhibitory activities of a series of commercially available or previously isolated phenolic acids, chlorogenic acids (CGAs) along with CGA synthetic derivatives (Fig. 2), were assessed. All derivatives used here are as well found in natural sources including many dietary plants or originating from food processing, in particular coffee roasting.[49] All derivatives tested were obtained from commercial sources or obtained by chemical synthesis and used in pure form with purity assessed by HPLC-MS. Two different NAs from *C. perfringens*, H5N1 and recombinant H5N1 (N-His)-Tag were chosen as suitable NAs. A fluorimetric assay established in the literature based on Amplex Red was used. The activity of the CGAs and their derivatives was compared directly with the inhibitory activities of commercial NAIs Tamiflu and Relenza as positive controls. Inhibitory concentrations chosen were at 10µM, 100µM and 1 mM with the lower concentrations in the range of dietary concentrations after intake of a cup of coffee. Determination of accurate inhibition constants was not possible due to the uncertain purity of the Neuroaminidases available. To allow adequate comparison of data, the activity of the compounds examined in this study was expressed relative to Tamiflu (Table 1). It has to be noted that the inhibitory data for Tamiflu

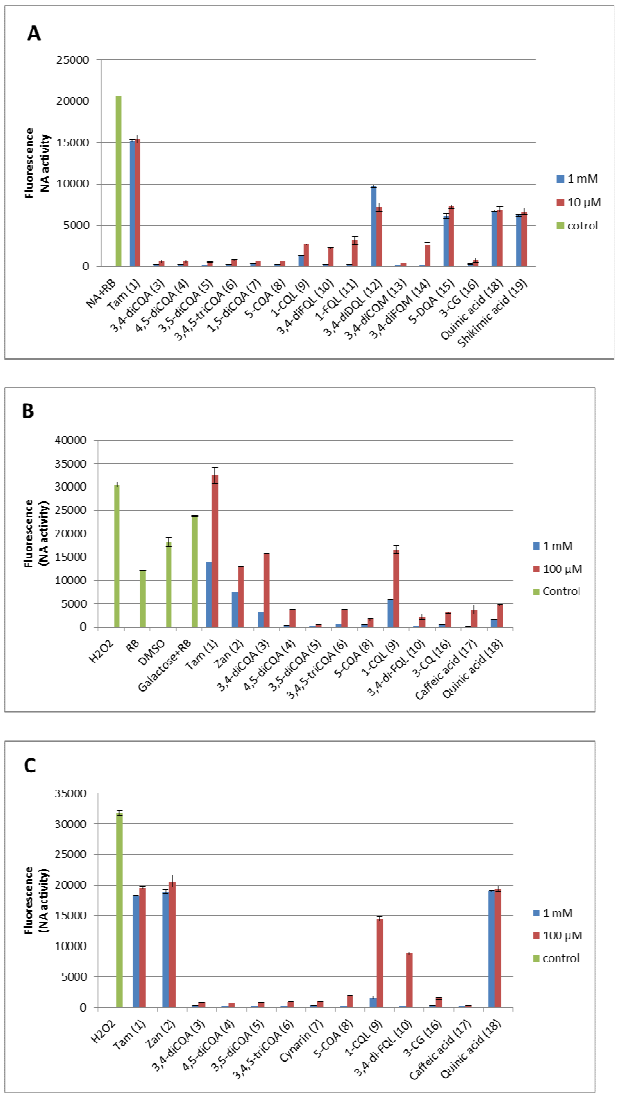

Fig. 3. Inhibitory activities of chlorogenic acids and their derivatives on neuraminidase from *C. perfringens* (**A**), H5N1 (**B**) and the recombinant H5N1 (N-His)-Tag (**C**)

and Relenza against the NAs used here are absent from the literature.

When comparing both NAs employed no significant difference in inhibition was observed (Fig. 3). We found that the activities of chlorogenic acids **3-8** were 40 – 70 times more active if compared to Tamiflu against the neuraminidase from *C. perfringens*, H5N1 and the recombinant H5N1 (N-His)-Tag. All inhibitory values were strongly dose-dependent (Table 1 and Fig 3). Mono caffeoyl derivative 8 showed similar activity if compared to dicaffeoyl derivatives **3-7**. In order to study the effect of the carboxylic acid group of quinic acid on the inhibitory activity, we synthesized chlorogenic acid lactones derivatives, compounds **9**-**12**. Interestingly, significant reductions of antiviral activities were observed for compound 9 against all tested NAs (Fig. 3). On the other hand, compound **12** showed low activity against NA from *C. perfringens*, although its activity was 12 times better than Tamiflu (Table 1). In contrast, compounds **10** and **11** showed relatively high activities against *C. perfringens* and H5N1 proteins. Moreover, compound **10** presented a very high activity against the recombinant virus protein H5N1. Compounds **11**-**15** were tested only against *C. perfringens* and not against H5N1 and the recombinant H5N1 (N-His)-Tag strains due to their insufficient quantities.

It is worth noting that four regioisomers of dicaffeoyl quinic acid show, within experimental error, very similar NA inhibitory activities.

These findings indicated that the 3,4-dihydroxyphenyl group from the caffeoyl was important for the activity,[23] while the carboxylic group of quinic acid had low influence on the compounds activities.

To further investigate the importance of hydroxyl groups of the caffeic acid, we synthesized compounds **13 – 15**. We found that compound **13** had grater activity against *C. perfringens* NA (Table 1 and Fig. 3A) due to the presence of the two catechol units. Methylation of two hydroxyl groups in 13 yielded compound **14** with reduced NA inhibitory activity especially in the low concentration range (10µM) (Fig. 2, 3 and Table 1). Compound **15**, which has no free hydroxyl group in the caffeoyl moiety, exhibited the lowest activity among the lactones derivatives. Again, these findings highlight the importance of the 1,2 catechol moiety for the activity.

In order to illustrate the influence of the caffeoyl moieties and to exclude the effects of the quinic acid part of chlorogenic acids and the synthesized chlorogenic acid lactones on their NA inhibitory activities, we further synthesized compound **16**, which has a glucose group connected to the caffeoyl moiety (Fig. 2). Interestingly, as shown in Table 1 and Fig. 3, the activity of this compound was similar to compound 8. This result confirmed that the activities of these compounds were mainly due to the 3,4-dihydroxyphenyl group from the caffeoyl moiety.[23, 50] For more evidence, we also examined the NA inhibitory activities of caffeic acid **17**, quinic acid **18** and shikimic acid **19**. The results showed that caffeic acid **17** displayed the NA inhibitory activity against H5N1 and recombinant H5N1 NAs, whereas quinic acid **18** and shikimic acid **19** did not supporting the notion of the caffeoyl group as main pharmacophore (Table 1 and Fig. 3).

In Table 1, it can be clearly seen that caffeic acid, chlorogenic acids and their derivatives were comparatively much more potent than the commercial antiviral drugs oseltamivir and zanamivir; this could be explained by the influence of divalent cations zinc (Zn^{2+}) or iron (Fe^{2+}) on the active center of the influenza enzyme, as previously reported.[51-55] Besides, the activity and stability of influenza virus neuraminidase is known to require other divalent cations such as calcium (Ca^{2+}) or magnesium (Mg^{2+}).[56-60] Moreover, Xu et al. demonstrated the presence of three Ca^{2+} binding sites in the influenza virus NA.[61] Thus, the observed NA inhibitory activities of these compounds may be related to coordination complexes between the metals and catechols [62] in the center of the active site.[63] We have recently shown that many

phenolic natural products exert enzyme inhibitory activity by a mechanism of non-selective allosteric denaturing of enzymes with multiple phenols binding to a single enzyme [64]. In this particular case, we are unable to rule out this mechanism since the purity of the commercial enzymes does not allow a reliable determination of Hill coefficients.

3.3 Relevance to the human diet

Many CGAs and derivatives, in particular those with a catechol moiety are able to inhibit viral Neuroaminidase at concentrations of 10µM or 100 µM. It is worth discussing these concentrations with respect to concentrations of CGAs after consumption of typical foods. The food richest in CGAs is coffee. A 200 ml serving of coffee contains on average 270 µmol mono caffeoyl quinic acids **8** (eg 120 µmol 5-CQA **8**) 60 µmol caffeoyl quinic acid lactones e.g. **9-12** and 15 µmol dicaffeoyl quinic acids **3-7**.[65] These concentrations imply that inhibition of viral NA will be relevant in the digestive system since concentrations far above the IC_{50} are obtained in the stomach and small intestine after consumption of a cup of coffee. Since caffeoyl quinic acids show low bioaivailibility and are intensly metabolized by the gut micro flora, only low nM concentratrions are observed in plasma and urine, therefore a systemic antiviral effect of these compounds is unlikely. Feruloyl quinic acids **11** and dimethoxycinnamoyl quinic acids show increased bioavailability, however, poor NA inhibition. The only class of compounds that might show relevance here are quinic acid lactones, which are formed during coffee roasting from CGAs. They have been reported to display 30 nM plasma T_{max} and 10 µmoles total urinary clearance, hence urinary concentrations approach concentrations required for NA inhibition. Additionally gut floral metabolites with a catechol moiety or structurally related dietary phenolics with a catechol moiety might serve as interesting compounds for future investigations.

4. Conclusions

In conclusion, we have shown that methanolic extracts of traditional Sudanese herbal medicines *A. maritima* and *S. oleraceus*, medicinal plants with recorded viral activity, are rich sources of phenolic compounds including chlorogenic acids, phenolic acids, flavonoids, flavonoids glycosides and coumarin derivatives. We have investigated the influenza virus NA inhibitory activities of naturally occurring chlorogenic acids against NAs from *C. perfringens*, H5N1 and recombinant H5N1 (N-His)-Tag. In comparison to the established NA inhibitors oseltamivir and zanamivir, all tested compounds show increased NA inhibitory activity in a fluorometric assay. There was no significant difference in inhibition between the different NA enzymes. Structure-activity studies suggest that the catechol unit within the caffeic acid moiety is required for NA inhibitory activity. The caffeic acid derivatives seem promising lead compounds for the development of antiviral drugs or suggest the use of plant material rich in chlorogenic acids as treatment for viral infections. Of these dietary plants in particular coffee and mate and members of the *Asteraceae* family such as artichoke or sunflower would offer promise. Dietary concentrations of CGA are sufficiently high to allow efficient NA inhibition in the digestive tract to suggest a relevance in human nutrition and disease prevention.

Abbreviations

5-CQA, 5-*O*-caffeoylquinic acid; 3,4-diCQA, 3,4-di-*O*-caffeoylquinic acid; 4,5-diCQA, 4,5-di-*O*-caffeoylquinic acid; 3,5-diCQA, 3,5-di-*O*-caffeoylquinic acid; 1,3-diCQA, 1,3-di-*O*-caffeoylquinic acid; 3,4,5-triCQA, 3,4,5-tri-*O*-caffeoylquinic acid; 1-CQL, 1-*O*-caffeoyl-1,5-quinide; 3,4-diFQL, 3,4-di-*O*-feruloyl-1,5-quinide; 1-FQL, 1-*O*-feruloyl-1,5-quinide; 5-DQA, 5-*O*-dimethoxycinnamoylquinic acid; 3,4-diDQL, 3,4-di-*O*-dimethoxycinnamoyl-1,5-quinide; 3,4-diCQM, methyl 3,4-di-*O*-caffeoylquinate; 3,4-diFQM, methyl 3,4-di-*O*-feruloylquinate; 3-CG, 3-*O*-caffeoylglucose.

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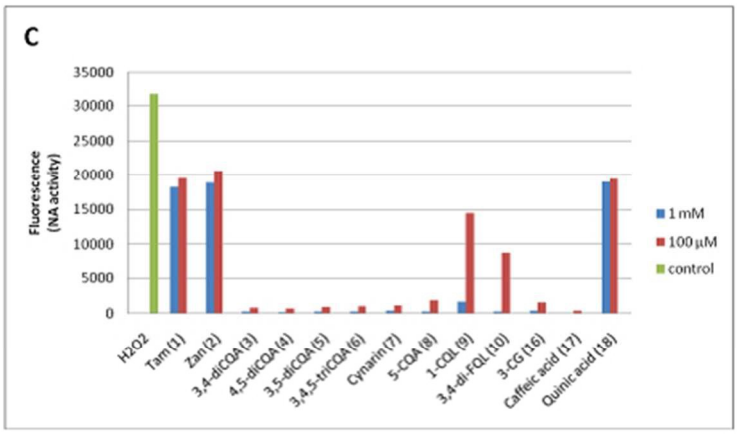

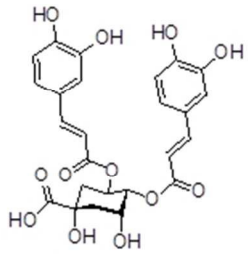

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