Bioorganic & Medicinal Chemistry Letters xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design, synthesis and exploring the quantitative structure–activity relationship of some antioxidant flavonoid analogues

Sreeparna Das [a], Indrani Mitra [b], Shaikh Batuta [a], Md. Niharul Alam [a], Kunal Roy [b,*], Naznin Ara Begum [a,*]

[a] Bio-Organic Chemistry Laboratory, Department of Chemistry, Siksha Bhavana, Visva-Bharati (Central University), Santiniketan 731 235, West Bengal, India
[b] Drug Theoretics and Cheminformatics Laboratory, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700 032, India

ARTICLE INFO

Article history:
Received 14 June 2014
Revised 2 September 2014
Accepted 3 September 2014
Available online xxxx

Keywords:
Flavonoids
Antioxidants
DPPH assay
QSAR
In silico method

ABSTRACT

A series of flavonoid analogues were synthesized and screened for the in vitro antioxidant activity through their ability to quench 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical. The activity of these compounds, measured in comparison to the well-known standard antioxidants (**29**–**32**), their precursors (**38**–**42**) and other bioactive moieties (**38**–**42**) resembling partially the flavone skeleton was analyzed further to develop Quantitative Structure–Activity Relationship (QSAR) models using the Genetic Function Approximation (GFA) technique. Based on the essential structural requirements predicted by the QSAR models, some analogues were designed, synthesized and tested for activity. The predicted and experimental activities of these compounds were well correlated. Flavone analogue **20** was found to be the most potent antioxidant.

Oxidative stress in human arises from an imbalance in the antioxidant status, that is, production of excessive Reactive Oxygen Species (ROS) during cellular metabolism versus living cell's own defense and repair mechanisms. This oxidative stress plays a crucial role in the age-associated diseases such as cardiovascular and cerebrovascular diseases, some forms of cancer and Parkinson's and Alzheimer's diseases.[1,2]

Antioxidants primarily reduce this oxidative stress. Endogenous antioxidants are produced in the living cells itself and they are the integral part of body's own defense systems. Various enzymes, for example, superoxide dismutase, catalase and glutathione peroxidase, vitamin E, uric acid and serum albumins represent this class of antioxidants. But consumption of exogenous antioxidants or dietary antioxidants (i.e., antioxidants obtained from dietary supplements) is also important to fight against oxidative stress and associated diseases in human beings.[2] Among the dietary antioxidants, flavonoids play substantial role besides ascorbate, tocopherols and carotenoids. Flavonoids represent one of the most diverse and extensively spread groups of plant derived natural products.[1,2] Moreover, these compounds can also be synthesized in the laboratory using simple synthetic methods and easy laboratory set-up.[3–5]

The quest for novel and non-toxic antioxidants with highly specified nutritional and therapeutic properties is an extremely important and challenging job. Over the past few years, a plethora of medicinal plants have been put to trial as the source of naturally occurring antioxidants. On the other hand, design and synthesis of new antioxidants is a highly cherished goal for researchers working in the field of 'Antioxidant Chemistry'. Moreover, it is extremely desirable that these synthetic products must be non-toxic and their synthetic protocols should be energy efficient, economically viable and environment friendly. But synthetic antioxidants like butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA) which are frequently used as preservatives in processed foods are found to be carcinogenic. Moreover these synthetic antioxidants show adverse effects on the lungs and liver.[6,7]

Flavones represent a major sub-class of flavonoid type of compounds which have structure consisting of two aromatic rings (A and B) linked by three carbons in an oxygenated heterocycle (Ring C)[8] (Fig. S1(a) in Supplementary data). Chalcones (Fig. S1(b) in Supplementary data) represent another sub-class of the flavonoid family.[8]

These flavonoids have wide spectrum of biological and pharmacological applications as cardioprotective, chemoprotective antimicrobial, antiviral, anti-allergic, hepato-protective and anticancer agents.[1,2,8] It is believed that most of these bioactivities of flavonoid type compounds may be originated due to their behavior as antioxidants.[1,2] The antioxidant effects of flavonoids are believed to be due to their (a) interactions with several enzymes; (b) ability to scavenge ROS and other free radicals; (c) ability to chelate metal ions and (d) synergistic effects when

* Corresponding authors. Fax: +91 3328371078 (K.R.); tel.: +91 9434431810; fax: +91 3463261526 (N.A.B.).
E-mail addresses: kroy@pharma.jdvu.ac.in (K. Roy), naznin.begum@visva-bharati.ac.in (N.A. Begum).

http://dx.doi.org/10.1016/j.bmcl.2014.09.028
0960-894X/

attached/added with other antioxidants.[1,2] Moreover, the antioxidant activities of these type of compounds are on an intimate terms with their structural pattern [i.e., nature and position of substituent(s) on Ring-A, B and C], geometry and physico-chemical parameters.[1–3,8] Although antioxidant activity of various flavone type of compounds have been well examined but there are few reports on their systematic study on structure–activity relationship and this type of systematic study on their antioxidant activity and molecular structures is of great importance to develop potential drugs which can be used to treat free radical associated diseases.

In the present work, we have synthesized a series of chalcone and flavone analogues by varying the substitution patterns on ring A and ring B or by varying the length of the conjugation pattern of the parent flavone moiety [structures are shown in Fig. S2(a and b) in Supplementary data]. Flavonoid analogues (both isolated and synthesized) along with their precursors and standard antioxidants were evaluated for in vitro antioxidant activity by their measuring 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) radical scavenging ability (Table 1).

We have also extended this work towards the development of Quantitative Structure–Activity Relationship (QSAR) models[9] on the basis of relevant physico-chemical, spatial and electronic properties of these compounds. Genetic Function Approximation (GFA) method[10] with linear and spline options was applied as the chemometric tool to develop the QSAR models which would give us a clear idea about the relationship between the antioxidant activity and the structural patterns of these compounds. This was further explored as our guiding tool for lead optimization. Based on the essential structural requirements for showing potent antioxidant activity as explored from the developed QSAR models, twenty flavonoid analogues were designed. Some of these designed compounds were synthesized and tested for their DPPH radical scavenging potential. The predicted activity of the designed molecules was found to be in accordance with experimental data predicted by in silico methods.

A total of forty two compounds (among which twenty three flavonoid analogues were synthesized presently) were tested for DPPH radical scavenging antioxidant activity[11,12] and their activity data was used to develop QSAR models.[9,10] Table 1 displays structures as well as the IC_{50} (sample concentration having 50% radical inhibition activity) values of these compounds. Three replicates were performed for each experiment. The results are expressed as mean ± standard deviation (SD).

Table 1
DPPH radical scavenging activity of flavonoid analogues and related compounds used for the development of QSAR models

Compound tested	DPPH Radical scavenging activity [(IC_{50} ± SD) (mM)]
(*E*)-1,3-Diphenyl-2-propen-1-one (**1**)[2]	3139.260 ± 8.620
(*E*)-3-(4-Nitrophenyl)-1-phenylprop-2-en-1-one (**2**)[1]	2815.900 ± 5.991
(*E*)-1-Phenyl-3-*p*-tolylprop-2-en-1-one (**3**)[2]	115.160 ± 2.425
(*E*)1-(2-Hydroxyphenyl)-3-phenylprop-2-en-1-one (**4**)[1]	77.986 ± 1.145
(*E*)-1-(2-Hydroxyphenyl)-3-*p*-tolylprop-2-en-1-one (**5**)[1]	7.723 ± 0.226
(*E*)-3-(4-Chlorophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**6**)[2]	6.103 ± 0.122
(*E*)-1-(2-Hydroxy-phenyl)-5-phenyl-penta-2,4-dien-1-one (**7**)[1]	7.133 ± 0.371
(*E*)-3-Furan-2-yl-1-(2-hydroxy-phenyl)-prop-2-en-1-one (**8**)[1]	4.273 ± 0.129
(*E*)-1-(2-Hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (**9**)[1]	190.000 ± 2.427
(*E*)-1,5-Diphenyl-penta-2,4-dien-1-one (**10**)[1]	113.000 ± 3.807
(*E*)-4-Phenylbut-3-en-2-one (**11**)[1]	302.180 ± 3.769
(*Z*)-3-Hydroxy-1-(4-methoxybenzofuran-5-yl)-3-phenylprop-2-en-1-one or pongamol (**12**)[2 a]	11.640 ± 0.375
2-Phenyl-4*H*-chromen-4-one (**13**)[1]	9590.000 ± 8.544
2-(4-Methoxyphenyl)-4*H*-chromen-4-one (**14**)[1]	681.783 ± 4.923
2-(4-Chlorophenyl)-4*H*-chromen-4-one (**15**)[1]	0.725 ± 0.052
2-*p*-Tolyl-4*H*-chromen-4-one (**16**)[1]	6.338 ± 0.334
3-Hydroxy-2-phenyl-4*H*-chromen-4-one (**17**)[1]	0.387 ± 0.004
2-(4-Chlorophenyl)-3-hydroxy-4*H*-chromen-4-one (**18**)[2]	0.663 ± 0.019
3-Hydroxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one (**19**)[2]	0.114 ± 0.004
3-Hydroxy-2-*p*-tolyl-4*H*-chromen-4-one (**20**)[1]	$0.005 \pm 8.5 \times 10^{-05}$
2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one (**21**)[1]	$0.011 \pm 9.6 \times 10^{-05}$
2-(4-(Dimethylamino)phenyl)-3-hydroxy-4*H*-chromen-4-one (**22**)[2]	0.022 ± 0.001
2-(Benzo [*d*][1,3]dioxol-5-yl)-3-hydroxy-4*H*-chromen-4-one (**23**)[1]	0.094 ± 0.004
3-Hydroxy-2-styryl-chromone-4-one (**24**)[2]	0.039 ± 0.001
2-Furan-2-yl-3-hydroxy-chromone-4-one (**25**)[1]	0.034 ± 0.002
3-Methoxy-2-phenyl-4*H*-furo[2,3-h]chromen-4-one or karanjin (**26**)[1 a]	4.232 ± 0.051
3,5,7-Trihydroxy-2-phenyl-4*H*-chromen-4-one or galangin (**27**)[2 b]	0.191 ± 0.006
5,7-Dihydroxy-2-phenylchroman-4-one or pinocembrin (**28**)[2 b]	59.300 ± 1.299
Gallic acid or 3,4,5-trihydroxybenzoic acid (**29**)[1]	0.011 ± 0.0002
Ascorbic acid or (*R*)-5-[(*S*)-1,2-dihydroxyl]-3,4-dihydroxyfuran-2(5*H*)-one (**30**)[1]	0.033 ± 0.0004
Trolox or 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (**31**)[1]	0.012 ± 0.0001
Butylated hydroxy anisole (BHA) or 2-*tert*-butyl-4-methoxyphenol or (**32**)[1]	0.019 ± 0.0001
Salicylic acid or 2-hydroxybenzoic Acid (**33**)[1]	278.303 ± 4.640
Cinnamic acid or (*E*)-3-phenylprop-2-enoic acid (**34**)[1]	1727.617 ± 7.142
p-Hydroxy cinnamic acid or (*E*)-3-(4-hydroxyphenyl)-2-propenoic acid (**35**)[1]	18.450 ± 0.679
Piperine or 1-[5-(1,3-Benzodioxol-5-yl)-1-oxo-2,4-pentadienyl] piperidine (**36**)[1]	90.693 ± 2.467
Carbazol-9-yl-methanol (**37**)[1,c]	$3.690X10^{9}$ ± 3.185
Piperonal or Benzo[*d*][1,3]dioxole-5-carbaldehyde (**38**)[1]	906.943 ± 4.121
Salicylaldehyde or 2-hydroxybenzaldehyde (**39**)[2]	96.650 ± 0.976
Cinnamaldehyde or (2*E*)-3-phenylprop-2-enal (**40**)[1]	372.167 ± 2.142
Furfural or furan-2-carbaldehyde (**41**)[2]	214.777 ± 1.275
ortho-Hydroxy acetophenone or 1-(2-hydroxyphenyl) ethanone (**42**)[1]	154.887 ± 1.798

[1] Training set compounds for QSAR modeling.
[2] Test set compounds for QSAR modeling.
[a,b] Previously isolated from seed extract of *Pongamia glabra* Vent. and ethanol extract of Indian propolis.[13–15]
[c] Synthesized according to the previously reported method.[15]