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Effects of thermal treatment, ultrasonication, and sunlight exposure on antioxidant properties of honey

Isıl işlem, ultrasonikasyon ve güneş ışığına maruz kalmanın balın antioksidan özelliklerine etkileri

Short Tittle:
Effects of some parameters on antioxidant quality of honey
Bazı parametrelerin balın antioksidan kalitesine etkileri

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ABSTRACT
INTRODUCTION: The aim of this study was to determine how the antioxidant capacity, total phenolic content and total flavonoid content change in honey after it was subjected to controlled heating, ultrasonication and sunlight.
METHODS: Honey was subjected to thermal treatment (for 5-20 min at 30-80°C), ultrasonication (for 5-20 min at 37 kHz frequency) and sunlight (for 1-10 days) to evaluate their impacts on antioxidant capacity, total phenolic and flavonoid contents. The one-way ANOVA followed by Tukey's test was performed to compare differences between experimental results.
RESULTS: Generally, antioxidant quality of samples heated at 60 and 80°C were negatively affected as compared to untreated samples ($p<0.05$); however, statistically significant differences between untreated samples and samples heated at 30 and 45°C were not found. On the other hand, ultrasonication of honey samples for 60 min showed enhancement in antioxidant properties compared to untreated samples ($p<0.05$). Also, while exposure to sunlight for 10 days caused decrease in total phenolic content value of honey, total flavonoid content and antioxidant capacity values started to decrease after 6 days ($p<0.05$).
DISCUSSION AND CONCLUSION: The results show that producers and consumers should consider the adverse effects of sunlight and temperature on antioxidative quality of honey. Also, ultrasonication technique has advantages in order to preserve antioxidant properties of honey.
Keywords: Honey, Temperature, Ultrasonication, Sunlight, Antioxidative quality
ÖZ
GİRİŞ ve AMAÇ: Bu çalışmanın amacı, kontrollü ısıtma, ultrasonikasyon ve güneş ışığına tabi tutulduktan sonra balın antioksidan kapasitesinin, toplam fenolik içeriğinin ve toplam flavonoid içeriğinin nasıl değiştiğini belirlemektir.

YÖNTEM ve GEREÇLER: Bal, antioksidan kapasite, toplam fenolik ve flavonoid içerikleri üzerindeki etkilerini değerlendirmek için ısıl işlem (30-80°C sıcaklıkta 5-20 dakika), ultrasonikasyon (37 kHz frekansta 5-20 dakika) ve güneş ışığına (1-10 gün arası) tabi tutuldu. Deneysel sonuçlar arasındaki farklılıkları karşılaştırmak için tek yönlü ANOVA ve Tukey testi yapıldı.
BULGULAR: Genel olarak, 60 ve 80 °C'de ısıtılan numunelerin antioksidan kalitesi, muamele edilmeyen numunelere kıyasla olumsuz etkilenmiştir ($p < 0.05$); bununla birlikte, muamele edilmemiş numuneler ile 30 ve 45 °C'de ısıtılmış numuneler arasında istatistiksel olarak anlamlı farklar bulunmamıştır. Öte yandan, bal numunelerinin 60 dakika ultrasonikasyonu, muamele edilmeyen numunelere kıyasla antioksidan özelliklerinde artış gösterdi ($p<0.05$). Ayrıca 10 gün güneş ışığına maruz kalmak balın toplam fenolik içerik değerinde düşüşe neden olurken, toplam flavonoid içeriği ve antioksidan kapasite değerleri 6 gün sonra düşmeye başladı ($p<0.05$).
TARTIŞMA ve SONUÇ: Sonuçlar, üreticilerin ve tüketicilerin güneş ışığının ve sıcaklığın balın antioksidan kalitesi üzerindeki olumsuz etkilerini dikkate alması gerektiğini göstermektedir. Ayrıca ultrasonikasyon tekniğinin balın antioksidan özelliklerini korumak için avantajları vardır.
Anahtar Kelimeler: Bal, Sıcaklık, Ultrasonikasyon, Güneş ışığı, Antioksidan kalite

This manuscript was presented in 8th Black Sea Basin Conference on Analytical Chemistry, 9-11 May 2018 in Istanbul. Its abstract published in the abstract book: Honey is a health promoting natural food due to its bioactive compounds such as phenolic acids and flavonoids. Antioxidant, antimicrobial, antitumoral, antiinflammatory and wound healing activities reveal its medicinal value (1-3). However, the quality of honey is the primary factor effects these health benefits. On the other hand, the quality of honey depends on various parameters such as floral origin, environmental conditions, industrial processing and storage.

Crystallization is one of the main problem during industrial processing and storage. Heat treatment is useful way to solve this problem. Ultrasonication is an alternative technique in order to prevent and delay crystallization (4). Exposing to sunlight is an important environmental condition during the period on the shelf.

In the present study, it is aimed to evaluate the effects of thermal treatment, ultrasonication and sunlight on antioxidant activity of honey, which is one of the quality parameters of honey. In brief, results indicated that thermal treatment at 60 and 80 °C, exposure to sunlight for 10 days negatively affected antioxidant properties of honey. However, ultrasonication for 60 min. promoted to the values.

INTRODUCTION

Honey is a natural product generated by honeybees and it has great market potential due to its health benefits for humans. Honey is well known as a source of enzymatic and non-enzymatic antioxidants such as glucose oxidase, catalase, phenolics, flavonoids, vitamins, proteins and Maillard reaction products[1]. Phenolics are the main components in honey which are attributed to its antioxidant activity[2]. However, the antioxidant activity varies depending on the floral source, seasonal and environmental factors[3].

Honey has the unique combination of components. This characteristic makes it valuable diet for its consumers. However, honey is not normally commercialised in its raw state, so that it is not suitable for large-scale marketing without further treatment [4,5]. Commercial honey processing includes controlled heating to destroy yeast that can cause unwanted fermentation during the product's shell life and to make liquefaction so as to obtain fluid, non-crystallised

product [4-6]. Besides thermal treatments, ultrasonication has been also used as an alternative to make honey marketable [7]. However, the effect of industrial processing on the antioxidant capacity of honey has not been studied in depth.
Honey is inevitably exposed to sunlight during the period from production to consumption. Sunlight exposure increases UV radiation. It is well known that UV radiation exposure adversely affects quality of foods [8], but there is no information on the effects of natural UV radiation on honey antioxidant properties.
Since the honey provides health benefits and the demand for high quality honey, the preservation and enhancement its antioxidant properties during the process and storage are considerably important. Therefore, the aim of this study was to determine how the antioxidant capacity, total phenolic content and total flavonoid content change in honey after it was subjected to controlled heating, ultrasonication and sunlight.

MATERIALS AND METHODS

Materials

Three bottle of same brand's honey were purchased from common chain market in Turkey. Honey brand chosen in this study is well known honey brand all over the Turkey. Brand officially declares that they own British Retail Consortium certificate and all chemical and physical analysis are performed to assure authenticity of honey.

Methods

Thermal treatment procedure

Samples were subjected to thermal processing in a water bath for 5, 10, 15 and 20 min separately at 30, 45, 60 and 80 °C. Afterwards, samples were cooled to room temperature for the determinations of antioxidant capacity, total phenolic and flavonoid contents.

Ultrasonication procedure

The sonication of samples were performed at 37 kHz frequency for 5, 15, 30 and 60 min, using an ultrasonic cleaning bath.

Exposing to sunlight

Samples were placed outdoor during daytime (Average max. temperature 26.0 °C) and nighttime (Average min. temperature 15.5 °C) in May for 1, 3, 6 and 10 days of sunlight exposure.

Analysis of antioxidant capacity

Cupric reducing antioxidant capacity (CUPRAC)

CUPRAC was determined as described by Apak et al.[9] 1 g of processed honey sample were dissolved in 2.5 mL distilled water. Then 0.1 mL of the solution were mixed with 0.75 mL copper (II) chloride (10mM), 0.75 mL neocuproine (7.5mM), 0.75 mL ammonium acetate buffer (1M, pH=7.0), and 0.75 mL of distilled water. After 30 min, absorbance was measured at 450 nm. Trolox was used as a reference standard. Results were expressed as µmol Trolox equivalent per one gram (µmol TE/g).

Trolox equivalent antioxidant capacity (TEAC)

The estimation of the TEAC was carried out based on the method of Re et al.[10] 0.1 mL of honey solution with the concentration of 1 g/2.5 mL were mixed with 2 mL of $ABTS^{\cdot+}$ (2,2'-azinobis-3-ethylbenzothiazoline-6- sulfonic acid) solution. After 15 min, absorbance was measured at 734 nm. The standard curve was constructed using Trolox and the results were expressed as µmol TE/g.

Total phenolic (TPC) and flavonoid contents (TFC)

TPC was assessed according to the Fu et al.[11] 0.1 mL of honey solution with the concentration of 1 g/2.5 mL were mixed with 1.0 mL of 1:10 diluted Folin-Ciocalteu reagent. After 4 min,

1.0 mL of saturated sodium carbonate solution (about 75 g/L) was added. This solution mixture was then incubated for 2 h at room temperature. The absorbance was measured at 760 nm after incubation. Gallic acid was used as standard to produce the calibration curve. Results were expressed as mg of gallic acid equivalent per 100 g (mg GAE/100g).

TFC was determined as described by Meda et al.[12] Briefly, 1.5 ml of 2% aluminium trichloride in methanol were mixed with the same volume of a honey solution (1 g/2.5 ml). After 10 min, absorbance was measured at 415 nm. A standard curve was obtained by using quercetin and results were expressed as mg of quercetin equivalent per 100 g (mg QE/100g).

Statistical analysis

Statistical analysis was carried out using GraphPad Prism 5 and Microsoft Excel Software. All experiments were conducted in triplicate. Analysis of variance (one-way ANOVA) was performed, and significant differences between mean values were determined by Tukey's multiple comparison test at a significance level of $p<0.05$.

RESULTS AND DISCUSSION

Effect of thermal treatment

Table 1 shows the antioxidant capacity, total phenolic and flavonoid contents of honey before and after heat treatment. The CUPRAC, TEAC, TPC and TFC values were found 2.75, 1.14 (μmol TE/g), 27.75 (mg GAE/100g), and 6.76 (mg QE/100g), respectively for untreated samples. It was found that samples heated at 30 °C and 45 °C for 5 min had the highest TPC and TFC values, respectively as compared to the rest. In the cases of CUPRAC and TEAC, the highest results obtained from untreated samples. On the contrary, TPC, TFC and antioxidant capacity values of samples were decreased with the increase of treatment of temperature. In order to assess if these differences have statistical significance, one-way ANOVA followed by Tukey's test was applied to the results. As seen in Table 1, statistical differences between untreated samples and samples subjected to 60 °C (in CUPRAC and TFC assays) and 80 °C heating (in all assays) were found significant. Also, findings revealed that process time as well as treatment temperature affected antioxidant capacity, total phenolic and flavonoid contents of samples.

Honey is rich in natural antioxidants such as enzymes, vitamins, phenolic acids and flavonoids [13]. However, these compounds may undergo many changes during thermal treatments [1]. Escriche et al.[4] evaluated the effect of industrial heat treatment on the phenolic compounds of Spanish honeys. According to their results, a significant decrease in the concentration of some phenolic compounds found in these honeys was determined after thermal treatment. Kowalski [14] investigated the impact of heating at 90 °C up to 60 min on antioxidant properties of honey using TPC and $ABTS^{\cdot+}$ assays. It was observed that there was a significant decrease in antioxidant properties of honeydew honey after processing. Chaikham et al.[15] reported that total phenolic and flavonoid contents and antioxidant capacity (measured by FRAP and DPPH assays) of longan-flower honey diminished after heating at 100 °C for 5 min.

Finally, heating honey at high temperatures could degrade antioxidant compounds [16] and this could explain why antioxidant capacity, TPC and TFC values of treated honey samples decrese in comparison with values of untreated samples in the present study.

Effect of ultrasonication

The results of impact of ultrasonication on antioxidant properties of honey are shown in Table 2. The values of treated samples increased with the increment of treatment time as compared to the values of untreated samples. However, statistical differences between samples ultrasonicated for 60 min and untreated samples in TPC, TFC and CUPRAC assays were determined significant. In the case of TEAC assay, significant differences between treated and untreated samples were not found. The differences between antioxidant capacity assays CUPRAC and TEAC were due to the different nature of the two assays [17,18].

Ultrasonication is an alternative and innovative technology to obtain fluid, non-crystallised product and it is more effective to preserve the nutrional values of honey than thermal treatments [7,15]. However, data on the impact of ultrasonication on antioxidant properties of honey are limited. Similar to current assay, Chaikham et al.[15] reported that TPC, TFC and antioxidant capacity values of honey increased after processing up to 20 min. Pollen is one of the important contents of honey [13]. It contains multiple essential components such as proteins, vitamins and phenolic compounds [19]. Ultrasonication has capability to increase the permeability of the plant tissues caused cell disruption resulting in liberation of all compounds present in cell [20]. In view of the fact that pollen is produced by plants as male cell, existing antioxidant compounds in pollen could be released after ultrasonication thereby causing increase in TPC, TFC and antioxidant capacity values of honey.

Besides limited studies relevant to the impact of ultrasonication on antioxidant properties of honey, many studies have been conducted to examine the influences of ultrasonication for preserving the nutrional qualities of fruit juices and its positive effects in terms of antioxidant properties have been demonstrated [21-23].

Effect of sunlight exposure

Table 3 displays the effects of sunlight exposure on TPC, TFC and antioxidant capacity of honey. TPC values of sunlight exposed samples started to change after 10 days, whereas exposure to sunlight caused change in TFC and TEAC values of samples after 6 days ($p<0.05$). However, CUPRAC values did not change significantly at any of samples compared with the untreated sample. Direct sunlight exposure initiates the generation of free radicals causing acceleration of degradation reactions that adversely affect quality of foods and beverages [8]. This could explain the decrease of TPC, TFC and antioxidant capacity values of honey. Until now there has been no research that would examine the influence of direct sunlight exposure on the antioxidant capacity and total phenolic and flavonoid contents of honey. However, several authors reported the sunlight induced quality loss of fruit products such as pummelo (*Citrus maxima*) essential oil [24] and strawberry juice [25].

CONCLUSIONS

The experimental results and statistical analysis indicated that treatments significantly affected the antioxidant properties of honey depending on process time. Thermal treatment and sunlight exposure negatively influenced the antioxidative quality of honey. However, ultrasonication significantly increased the values in all assays, except TEAC assay, where the increment was not found statistically significant. Therefore, ultrasonication could be an alternative technique in order to preserve antioxidant properties of honey instead of industrial thermal treatment. On the other hand, it is suggested that producers and consumers should consider the negative effects of sunlight on antioxidants of honey during the storage so that sunlight exposure has resulted in a decrease in antioxidant capacity, total phenolic and flavonid contents.

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Supplementary Information

In silico modeling of 4-(2-fluorophenoxy) quinoline derivatives as c-Met inhibitors in the treatment of human tumors

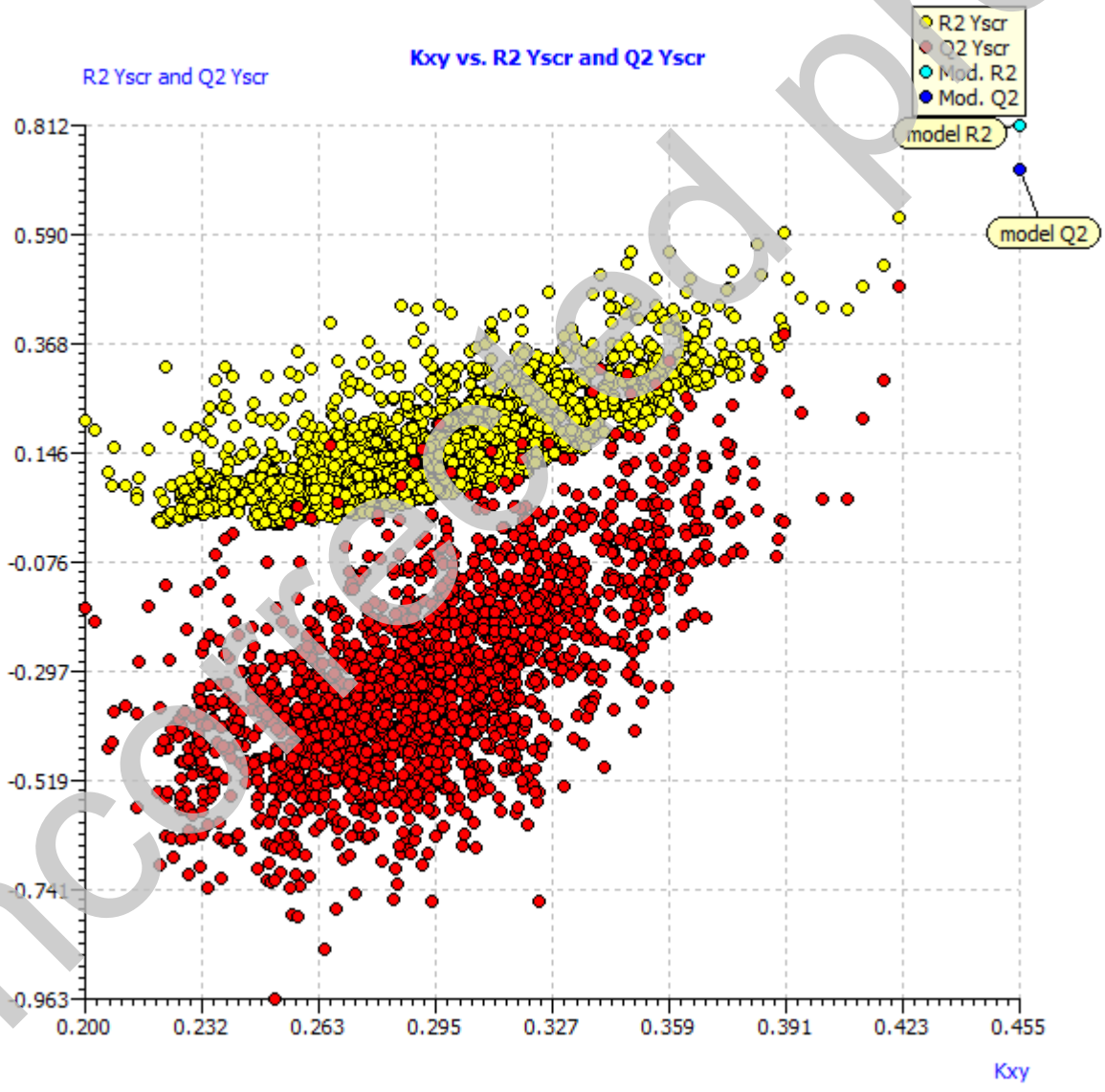


Figure SI. R^2 and Q^2 values belong to randomized models and the developed model.

Table SI1. Predicted values, leverages, and standardized residuals for the studied compounds.

Name	Prediction	Leverage (h*=0.577)	Std. Res.
10a	-1.698	0.318	0.163
10b*	-1.551	0.377	0.163
10c	-1.778	0.450	-0.697
10d	-1.262	0.354	0.650
10e	-1.251	0.171	0.229
10f	-1.605	0.213	-0.760
10g*	-1.602	0.362	0.920
10h	-0.968	0.150	-0.273
10i	-1.382	0.089	-1.672
10j	-1.425	0.138	-0.551
10k	-1.399	0.270	0.987
10l	-1.253	0.141	0.076
10m	-0.615	0.207	-1.354
10n	-0.579	0.218	0.588
10o	-0.564	0.229	-0.427
10p	-1.02	0.094	-0.882
10q	-0.954	0.170	-0.359
10r*	-0.927	0.218	-0.100
10s	-1.100	0.089	0.958
10t	-1.286	0.065	1.584
10u	-1.012	0.102	1.503
10v	-1.404	0.124	0.655
10w	-1.420	0.108	1.226
10x	-1.746	0.351	0.526
10y	-1.722	0.267	-0.326
11a	-1.311	0.175	-1.763
11b*	-1.216	0.148	0.301
11c*	-1.552	0.134	-1.415
11d*	-1.641	0.208	-0.933
11e	-1.604	0.101	-1.252
11f	-1.288	0.302	1.802
11g	-1.051	0.101	-0.531
D01	-0.797	0.253	-
D02	-0.429	0.507	-
D03	-0.898	0.370	-
D04	-0.863	0.284	-
D05	-0.997	0.395	-
D06	-0.535	0.607	-
D07	-0.191	0.716	-
D08	-1.468	0.254	-
D09	-0.801	0.457	-
D10	-1.454	0.284	-
D11	-1.112	0.305	-
D12	-1.787	0.279	-

D13	-2.306	0.574	-
D14	-2.310	0.571	-
D15	-1.276	0.549	-
D16	-1.427	0.361	-
D17	-1.299	0.463	-
D18	-1.404	0.430	-
D19	-1.345	0.417	-
D20	-1.456	0.336	-

* Test set

Table SI2. Descriptor values for the training, test set compounds and the designed compounds.

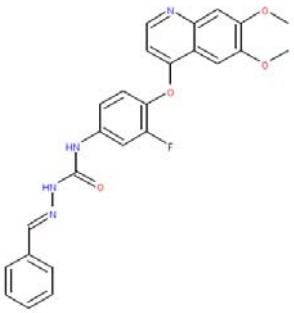
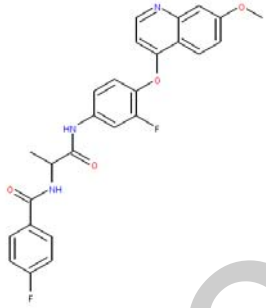
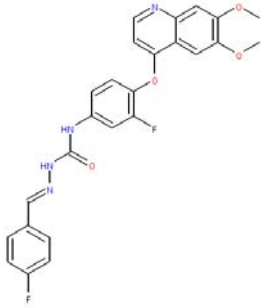
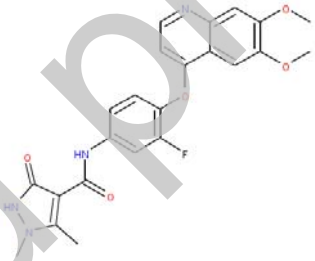
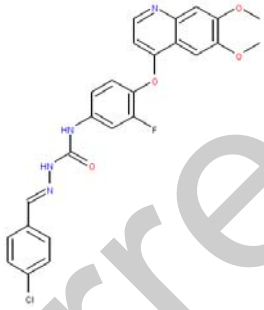
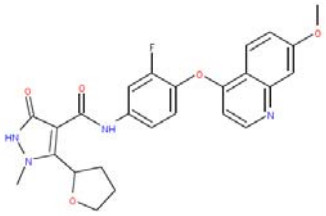
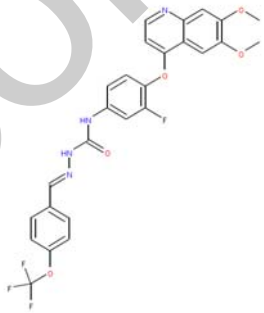
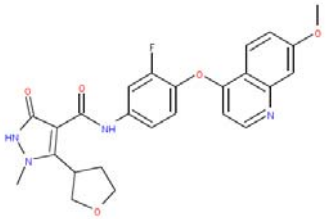
Name	PEOEVSA2	AATSC4m	SpMin8_Bh(e)	VR2_RG
10a	4.39	-1.448	1.117	1.041
10b*	9.185	-6.141	1.117	1.046
10c	9.185	-6.133	1.117	1.055
10d	9.185	-5.718	1.079	1.043
10e	9.185	-1.940	1.116	1.039
10f	9.185	1.331	1.153	1.049
10g*	9.185	-1.580	1.116	1.054
10h	9.185	5.164	1.116	1.038
10i	9.185	4.806	1.193	1.036
10j	9.185	3.149	1.193	1.035
10k	9.185	3.522	1.207	1.032
10l	9.185	5.772	1.194	1.032
10m	13.575	4.288	1.116	1.038
10n	13.575	5.375	1.116	1.038
10o	13.575	5.850	1.116	1.038
10p	9.185	3.471	1.116	1.038
10q	9.185	5.554	1.116	1.038
10r*	9.185	6.371	1.116	1.038
10s	9.185	1.195	1.116	1.038
10t	9.185	3.450	1.168	1.036
10u	9.185	3.799	1.116	1.038
10v	9.185	6.716	1.193	1.039
10w	9.185	6.203	1.193	1.039
10x	4.39	6.468	1.194	1.036
10y	9.185	3.803	1.194	1.048
11a	8.781	4.836	1.195	1.031
11b*	8.781	7.664	1.194	1.032
11c*	8.781	5.384	1.195	1.042
11d*	8.781	5.055	1.194	1.045
11e	8.781	3.151	1.195	1.041
11f	13.171	4.212	1.194	1.045
11g	8.781	3.572	1.118	1.037
D01	14.611	-1.569	1.091	1.047
D02	19.001	-3.687	1.081	1.047

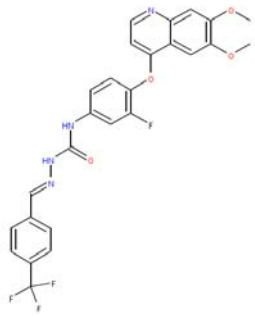
D03	14.611	-5.705	1.084	1.047
D04	14.611	-2.155	1.113	1.043
D05	14.611	-5.199	1.115	1.044
D06	4.390	-3.457	0.935	1.034
D07	4.390	-2.781	0.861	1.038
D08	8.781	-6.805	1.096	1.045
D09	8.781	-6.624	0.963	1.049
D10	8.781	-6.874	1.103	1.043
D11	8.781	-6.806	1.027	1.046
D12	4.390	-4.183	1.089	1.047
D13	4.390	2.018	1.153	1.062
D14	4.390	1.898	1.153	1.062
D15	4.390	-3.931	0.982	1.052
D16	4.390	-1.943	1.050	1.045
D17	4.390	2.410	1.052	1.046
D18	4.390	-3.237	1.025	1.048
D19	4.390	0.632	1.050	1.045
D20	4.390	-1.276	1.062	1.044

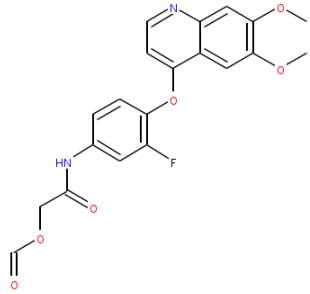
Table SI3. Toxicity predictions for the studied compounds.

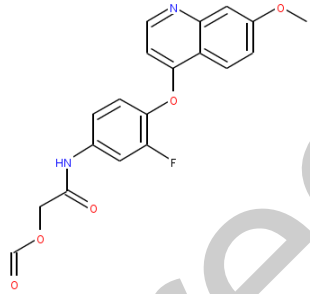
Software		SwissADME		pkCSM	
No	Name	PAINS	Brenk	Oral rat chronic toxicity (LOAEL) mg/kg_bw/day	Ratio (LOAEL/IC50)
1	10a	0	0	8.954	0.17
2	10b	0	0	8.954	0.24
3	10c	0	0	0.518	0.01
4	10d	0	0	2.965	0.13
5	10e	0	0	13.772	0.71
6	10f	0	0	2.698	0.09
7	10g	0	0	1.563	0.03
8	10h	0	0	3.076	0.37
9	10i	0	0	3.548	0.29
10	10j	0	0	2.158	0.10
11	10k	0	0	1.486	0.04
12	10l	0	0	1.489	0.08
13	10m	0	0	2.716	1.09
14	10n	0	0	1.972	0.42
15	10o	0	0	2.495	0.80
16	10p	0	0	2.104	0.28
17	10q	0	0	2.070	0.26
18	10r	0	0	2.158	0.27
19	10s	0	0	2.056	0.11
20	10t	0	0	1.114	0.03
21	10u	0	0	1.416	0.08
22	10v	0	0	2.495	0.08
23	10w	0	0	5.689	0.13
24	10x	0	0	10.965	0.16
25	10y	0	0	0.603	0.01
26	11a	0	0	52.723	5.04
27	11b	0	0	75.683	4.10
28	11c	0	0	39.355	1.92
29	11d	0	0	0.265	0.01
30	11e	0	0	0.237	0.01
31	11f	0	0	0.237	0.01
32	11g	0	0	66.069	7.24

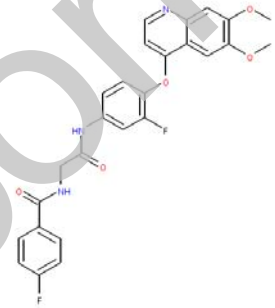
Table SI4. Molecular structures of designed compounds.

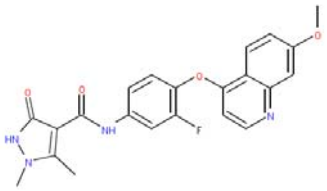
No	Name	Structure	No	Name	Structure
1	D01		11	D11	
2	D02		12	D12	
3	D03		13	D13	
4	D04		14	D14	

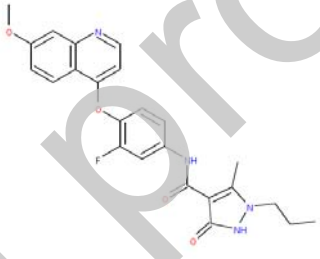
5 D05 

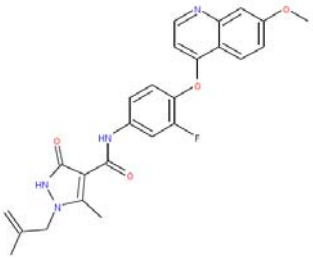
6 D06 

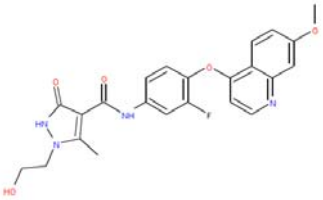
7 D07 

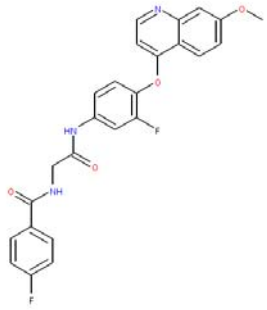
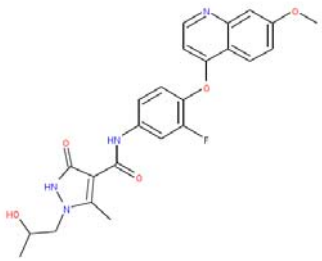
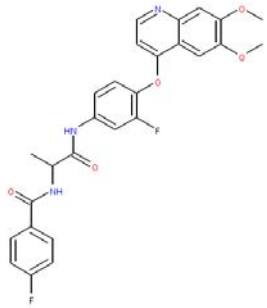
8 D08 

15 D15 

16 D16 

17 D17 

18 D18 

9	D09		19	D19	
10	D10		20	D20