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A QSAR Analysis of a Series of Acridone Derivatives Acting as HCV Inhibitors

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Abstract: Hepatitis C virus (HCV) was first identified in 1981. It has affected nearly 200 million people worldwide. The current treatment regimen has severe side effects and it demands new and effective drugs. In drug design and development, QSAR (Quantitative Structure-Activity Relationship) studies are of great help as they rationalize the drug synthesis and explain drug-receptor interactions. We performed a QSAR analysis on a data set of 33 acridone derivatives acting as HCV RNA replication inhibitors. The obtained correction has shown significant contribution of calculated molar refractivity, and hydrophobicity with r_{cv}^2 **= 0.87.** These results can be used to design novel scaffolds acting as anti-HCV agents.

Key word: HCV, QSAR, multiple regression analysis, correlation coefficient, CMR.

Introduction

Hepatitis C virus (HCV) was first identified in 1989 [1]. Hepatitis C virus was formally known as non-A and non-B hepatitis and the virus is transmitted primarily by blood and blood products [2,3]. HCV is an important human pathogens causing chronic hepatitis, cirrhosis of the liver, and hepatocellular carcinoma [4]. Nearly 200 million people are infected worldwide and up to 80% of them turn chronic infections [5].

HCV is a small; enveloped single standard (ss) RNA hepacivirus in the flaviviridae family. The HCV strains are classified in six major types and at least 30 subtypes [6]. The large number of HCV genotypes and quasi species is the main problem behind vaccine development for HCV. IFN-α, a naturally occurring glycoprotein and has antiviral, immunomodulatory properties and continues to be the only drug to induced sustained HCV clearance [7]. IFN-α monotherapy is limited by adverse side effect such as severe flu like syndrome, leucopenia and thrombocytopenia. Further more a sustained virological response is achieved in only 15 % of patients [8]. The combination of orally active ribavirin with IFN-α2b and amantidine has proved to be more effective than IFN-α monotherapy, yielding an sustained virological response in 35-40 % patients [9].

HCV is a positive, single strand RNA genome comprises of three regions i.e. 5' non translated region (NTR), a single open reading frame (ORF), of ~9,000 nucleotides, and a short 3' NTR (figure. 1). The 5'-NTR (~340 nucleotide) contains internal ribosome entry site (IRES), which mediate the initiation of viral RNA translation in a cap-independent manner. Sequences in the 5'NTR, including the IRES are essential for replication of this virus [10]. A short 3'NTR (230 nucleotide) comprises a tripartite structure having 3x sequence and the 3x region is crucial for efficient RNA replication [11]. A single open reading frame(ORF), is cleaved by host single peptidases and HCV-

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encoded protease to produce at least three structure proteins namely core protein, E1 protein, E2 protein and six non-structural proteins NS2, NS3, NS4A, NS4B, NS5A, and NS5B [12]. The core protein interacts with viral RNA and form the nucleocapsid. E1and E2 is heavily glycosylated viral envelope protein that can interact with the plasma membrane of hepatocytes and other cell [13]. The non-structural proteins encode enzyme or necessary factors that catalyze and regulate the replication of the HCV RNA genome. The NS2-NS3 zinc-dependent metalloproteinase undergoes autocatalytic cleavage to produce NS2 and NS3 [14].

The release of NS3 serine protease catalyses the cleavage of remaining NS polyprotein to yield NS4A, NS4B,NS5A and NS5B, which, together with NS3 and possibly cellular proteins, form the replication complex or 'replicase' of HCV. The carboxy- terminal segment of the NS3 protein also has nucleoside triphosphatase (NTPase) and RNA helicase activity. The NS4A protein acts as a cofactor for NS3 protease activity and the function of NS4B is not known. NS5A also function to regulate viral replication through its interaction with the NS5B protein [15], which is the RNA-dependent RNA polymerase (RdRp) that catalyses the replication of HCV RNA (Figure. 1).

Life Cycle of HCV

Virus binding and internalization (a); cytoplasmic release and uncoating (b); IRES-mediated translation and polyprotein processing (c); RNA replication (d); packaging and assembly (e); virionmaturation and release (f). The topology of HCV structural and non-structural proteins at the endoplasmic reticulum membrane is shown schematically. HCV RNA replication occurs in a specific membrane alteration, the membranous web. Note that IRES-mediated translation and polyprotein processing, as well as membranous web formation and RNA replication, which are illustrated here as separate steps for simplicity, might occur in a tightly coupled fashion. IRES, internal ribosome entry site (Fig. 2) [16].

Materials and methods

Data set of compouns

In a recent communication, Manfroni *et al.* [20] reported the series of 33 acridone derivatives (Table 1) acting as potential inhibitors of Hepatitis C virus RNA replicationThe biological activity value of these compounds was expressed as EC_{50} (μg/mL)and it is the ability to inhibit HCV replication with INF-α as positive control. Then, the EC50 values in μM/ml were converted to -logEC_{50} (pEC_{50}) to use in multiple regression analysis and discussion.

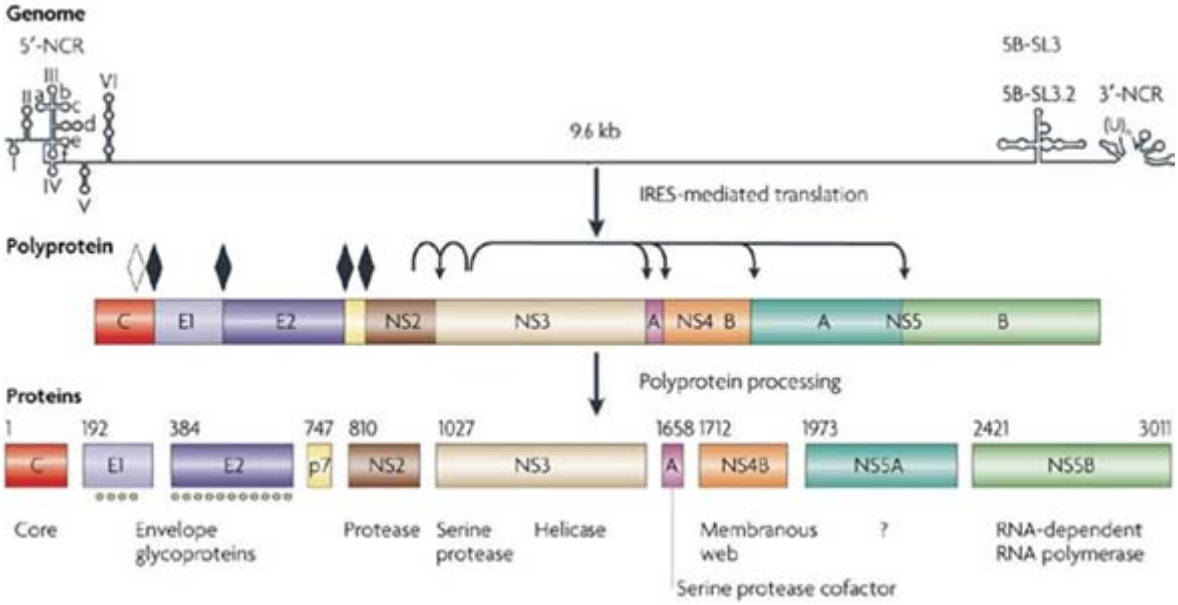


Figure 1. Schematic representation of the HCV genome and encoded viral protein [16]

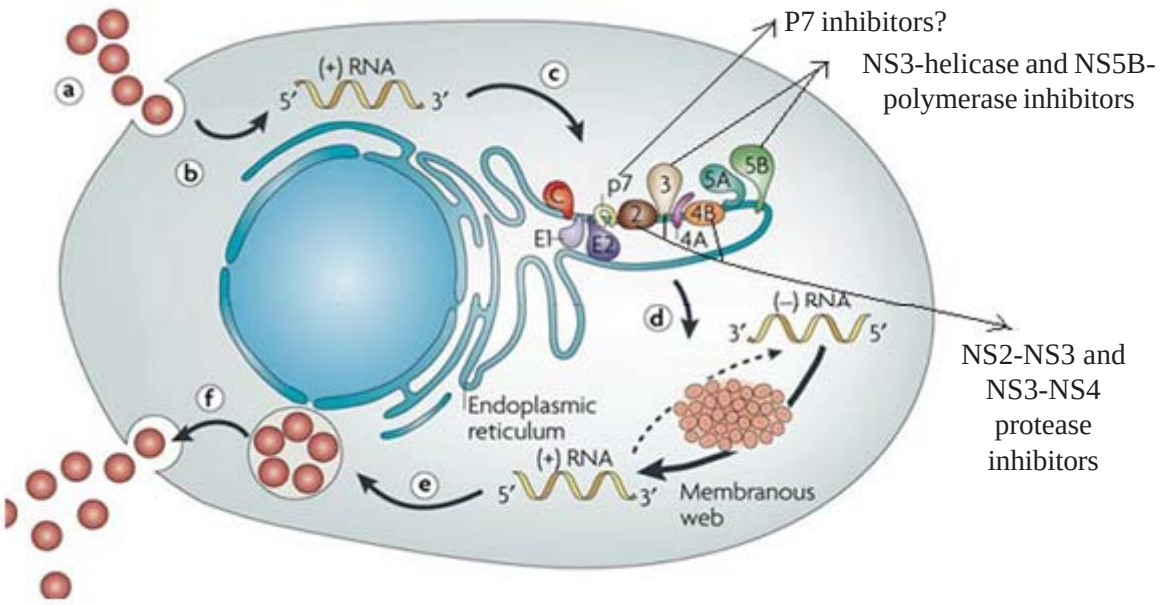
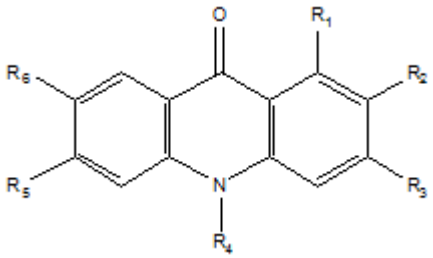


Figure 2. Life cycle of HCV showing virus binding and internalization (a); cytoplasmic release and uncoating (b); IRES-mediated translation and polyprotein processing (c); RNA replication (d); packaging and assembly (e); virion maturation and release (f). The topology of HCV structural and non-structural proteins at the endoplasmic reticulum membrane is shown chematically [16]

Table 1. Structures of Acridone Derivatives



Acridone basic nucleus

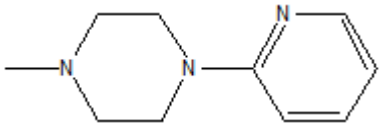
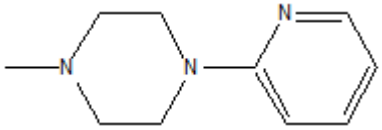
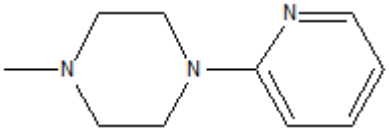
No	R^{I}	R^{II}	R^{III}	R^{IV}	R^{V}	R^{VI}
1	OMe	H	OMe	Me		NH_2
2	H	H	OMe	Me		NH_2
3	OMe	H	H	Me		NH_2

table 1. (continued).

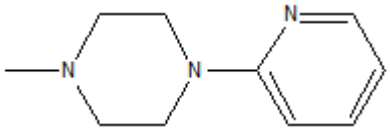
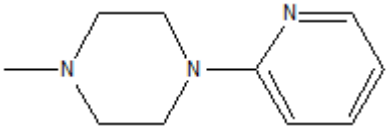
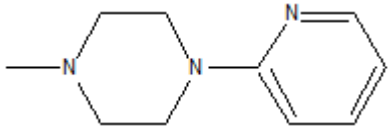
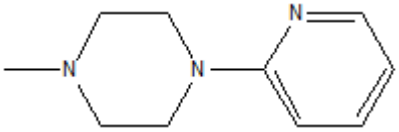
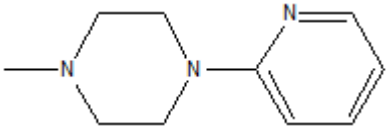
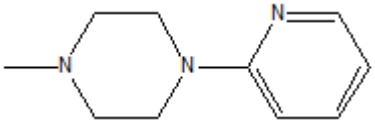
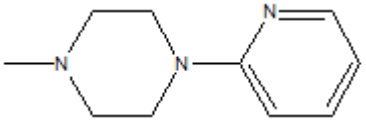
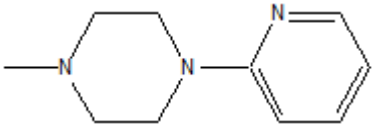
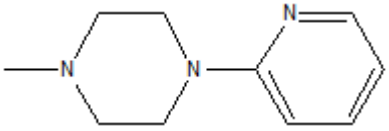
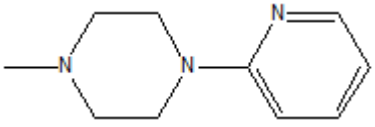
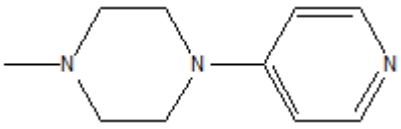
No	R^{I}	R^{II}	R^{III}	R^{IV}	R^{V}	R^{VI}
4	H	OMe	OMe	Me		NH_2
5	H	H	H	Me		NH_2
6	OMe	H	Me	Me		NH_2
7	OEt	H	OMe	Me		NH_2
8	OPr	H	OMe	Me		NH_2
9	Oi-Bu	H	OMe	Me		NH_2
10	OCH_2CO_2H	H	OMe	Me		NH_2
11	OMe	H	OMe	H		NH_2
12	OMe	H	OMe	Et		NH_2
13	OMe	H	OMe	*i*-Pe		NH_2
14	OMe	H	OMe	Me		NH_2

table 1. (continued).

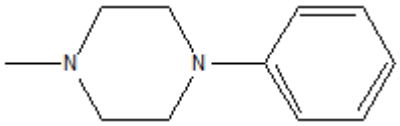
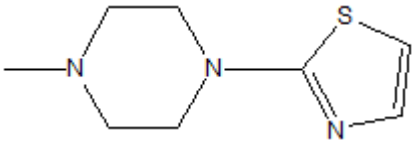
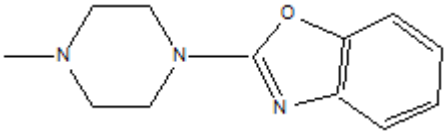
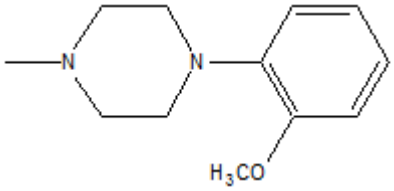
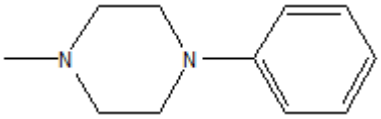
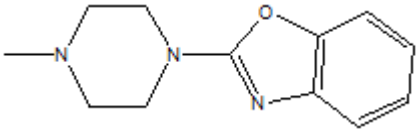
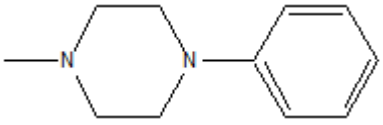
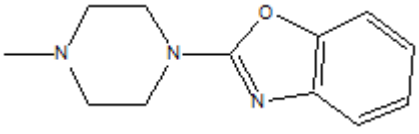
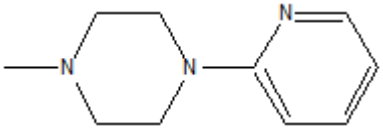
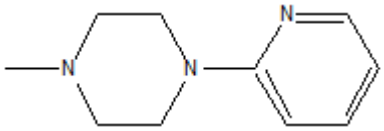
No	R^{I}	R^{II}	R^{III}	R^{IV}	R^{V}	R^{VI}
15	OMe	H	OMe	Me		NH_2
16	OMe	H	OMe	Me		NH_2
17	OMe	H	OMe	Me		NH_2
18	OMe	H	OMe	Me		NH_2
19	OMe	H	OMe	Me	OMe	NH_2
20	OH	H	OMe	Me		NH_2
21	OH	H	OMe	Me		NH_2
22	OH	H	OMe	Me	OMe	NH_2
23	OH	H	OH	Me		NH_2
24	OH	H	OH	Me		NH_2
25	OH	H	OH	Me	OMe	NH_2
26	OMe	H	OMe	Me		$N(Me)_2$

table 1. (continued).

No	R^I	R^{II}	R^{III}	R^{IV}	R^V	R^{VI}
27	OMe	H	OMe	Me		H
28	OMe	H	OMe	Et	Cl	NH_2
29	OH	H	OMe	Et	Cl	NH_2
30	OH	H	OH	Et	Cl	NH_2
31	OMe	H	OMe	H	Cl	NH_2
32	OH	H	OMe	H	Cl	NH_2
33	OH	H	OH	H	Cl	NH_2

Result and discussion

Multiple regression analysis

The various physicochemical parameters that are listed in the table 2, such as calculated molar refractivity (CMR) has been evaluated using ChemDraw version 8.0 software and π refers to the hydrophobic constant of the R^I substituents. The log EC_{50} was used as dependent variable, thus correlates the data linear to the free energy change.

When a multiple regression analysis was performed on the data of Table 1, it revealed the following correlation.

$$-\log EC_{50} = -0.257(\pm0.172)\ CMR + 0.282(\pm0.249)\ \pi - 1.067(\pm0.803)I_{Rv} + 8.511(\pm2.245)$$

$$n = 28\ r = 0.963\ r_{cv}^2 = 0.867\ s = 0.276\ F_{3,28} = 3.715 \quad \text{Eq. (1)}$$

In Eq. (1), n is the number of data points, r is the correlation coefficient, r_{cv}^2 is the square of the cross-validated correlation coefficient obtained from leave-one-out (LOO) jackknife procedure, s is the standard deviation, F is the F-ratio between the variances of calculated and observed activities, and the data within the parentheses with ± sign are 95 % confidence intervals. The figure within the parenthesis for F is the standard F-value at 99 % level. This equation represents a highly significant correlation between the HCV inhibition potency of the compounds and π values of R^I substituents and CMR of the compounds. Compounds 1, 2, 19, 20 and 26 were excluded during multiple regression analysis.

The Eq. (1) suggests that HCV inhibitory activity of the compounds will increase with the decrease in molar refractivity of the molecules and the hydrophobic R^I substituent would be advantageous to the HCV- inhibition potency.

There is also one indicator parameter I_{Rv} in this equation, where the former has been used with a value of 1 for R_V substituent being OMe and the value of 0 for other substituent.

A negative coefficient of I_{Rv} suggests that an OMe group at Rv-position will be detrimental to the activity. The negative effect of OMe group probably may be due to its involvement in the intramolecular hydrogen bonding with R_{VI}-substituents, which otherwise would form the hydrogen bonds with the receptors.

Conclusion

The results and discussion as presented above leads to the conclusion that anti-HCV potency of Acridone series of compounds can be governed by different physicochemical and structural properties. The physicochemical nature to permit all sorts of important interaction such as steric and hydrophobic interaction. In this study, predict logs (1/EC50) of some Acridone derivatives (1, 2, 22, 29, and 30) compounds are predicted to found to be essential for the activities of the series of inhibitors.

The results of multiple regression analysis and the binding mode of compounds can be used as a novel scaffold for anti-HCV for the further reconstruction and design of new protease inhibitors.

Table 3. Anti-HCV Activity and Physicochemical Parameters of Acridone derivatives (1-33)

No.	CMR	π	IRv	EC50 (μg/mL)	log (1/EC50) (Eq. 1) OBSD	CACL	PRED
1*	12.85	-0.02	0	1.6	5.800	5.217	5.689
2*	12.23	0.00	0	0.8	6.100	5.182	5.728
3	12.23	-0.02	0	12.5	4.900	5.367	5.235
4	12.85	0.00	0	6.0	5.220	5.213	5.156
5	11.61	0.00	0	4.0	5.400	5.531	5.403
6	12.54	0.56	0	5.0	5.300	5.451	5.226
7	13.31	0.39	0	4.0	5.400	5.205	5.384
8	13.78	0.26	0	17.0	4.770	5.048	5.020
9	14.24	1.66	0	3.8	5.420	5.324	5.227
10	13.50	-0.79	0	35.0	4.460	4.823	4.931
11	12.38	-0.02	0	4.0	5.400	5.328	5.242
12	13.31	-0.02	0	4.0	5.400	5.089	5.149
13	14.70	-0.02	0	10.0	5.000	4.732	5.134
14	12.85	-0.02	0	5.5	5.260	5.207	5.435
15	13.06	-0.02	0	9.0	5.050	5.153	5.143
16	12.66	-0.02	0	3.0	5.520	5.256	5.110
17	13.75	-0.02	0	27.0	4.570	4.976	4.877
18	13.68	-0.02	0	16.0	4.800	4.994	5.102
19*	8.75	-0.02	1	9.0	5.050	4.998	5.778
20*	12.60	-0.67	0	2.0	5.690	5.425	5.882
21	13.29	-0.67	0	50.0	4.300	5.192	5.339
22	8.75	-0.67	1	18.0	4.740	5.088	4.984
23	12.13	-0.67	0	4.4	5.360	5.009	5.025
24	12.82	-0.67	0	17.0	4.770	5.209	5.003
25	7.82	-0.67	1	5.0	5.300	5.032	5.331
26*	13.48	-0.02	0	1.0	6.000	5.122	5.813
27	12.48	-0.02	0	2.3	5.640	5.248	5.435
28	9.09	-0.02	1	5.0	5.300	5.302	5.413
29	8.62	-0.67	1	4.5	5.350	5.105	5.335
30	8.16	-0.67	1	5.0	5.300	5.042	4.894
31	8.16	-0.02	1	5.7	5.240	5.161	5.302
32	7.70	-0.67	1	8.0	5.100	5.344	5.382
33	7.23	-0.67	1	19.0	4.720	5.279	4.786

*****compounds were excluded.

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