

Synthesis of Analogues of Arbidol and Their Anti-viral Studies Towards Chikungunya Virus

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Abstract: *The fusion of virus and endosome membranes is an essential early stage in chikungunya virus infection. The low p^H -induced conformational change which promotes the fusogenic activity of the haemagglutinin (HA) is thus an attractive target as an antiviral strategy. The anti-chikungunya drug, arbidol is representative of a class of antivirals which inhibits HA-mediated membrane fusion by increasing the acid stability of the HA. In this study two series of indole derivatives structurally related to Arbidol were designed and synthesized to further probe the foundation of its antiviral activity and develop the basis for a structure–activity relationship (SAR). Ethyl 5-(hydroxymethyl)-1-methyl-2-(phenylsulphanymethyl)-1H-indole-3-carboxylate was identified as one of the most potent inhibitors and more potent than Arbidol against certain subtypes of chikungunya viruses..*

Keywords: Arbidol Derivatives, Antiviral Action, Chikungunya, Indole Analogues

I. INTRODUCTION

Chikungunya infection is commonly an acute disease marked by febrile arthralgia and a frequent rash: persisting arthralgia has been reported in a significant number of cases.¹ Chikungunya virus (CHIKV) is an arthropod-borne viral disease, this is usually found in tropics and hence the reason why Chikungunya is predominantly seen in Asian countries. First described in Tanzania in 1952 which has reappeared since 2005 in Eastern Africa, the Indian Ocean, India and South-East Asia and even reached Europe in 2007.² From 2005, this new variant has been responsible for millions of cases of CHIKV disease. Lethal infections are rare but severe cases have been described including neurological presentations and neonatal contaminations which were documented during the outbreak in Reunion Island.³ Current treatments of Chikungunya fever are for symptoms with no effective licensed vaccine nor specific antiviral drug available. The utilization of the antimalarial chloroquine proved to be poorly active *in vivo* despite it's in cellulo antiviral effect on CHIKV infection.^{4,5} Similarly, it has been shown that the combination of interferon-alpha and ribavirin is effective on CHIKV replication *in vitro* but these compounds have not been tested in animal models and/or clinical trials.⁶⁻¹⁰

II. PRESENT WORK

By its tropism for membranes and its inhibitory effect on viruses entry, fusion and replication, Arbidol opens promising perspectives in the search for new and efficient antiviral compounds,¹⁴ but since it has a relatively high CC_{50} value and it means toxic, its clinical application is forbidden.¹² So with the aim to reduce its toxicity and improve its antiviral properties a series of novel arbidol derivatives were designed and synthesised focusing our attention on different positions of the indole nucleus (Figure 1).

First of all the *N,N*-dimethylamino methyl group in position 4 was removed (compound **1** and **2**). Then phenolic OH in position 5 was homologated in hydroxyl methyl group (compounds **3** and **6**) and subsequently with the aim to investigate the role of phenyl sulfanyl methyl group on the antiviral activity, we introduced a methyl group at the position 2 (compounds **4** and **5**).

In addition, to evaluate the role of hydroxy group of compounds **3** and **4** in position 5, a new library of compounds was prepared replacing this group with several aliphatic amines and cyclic amines (Table 1).

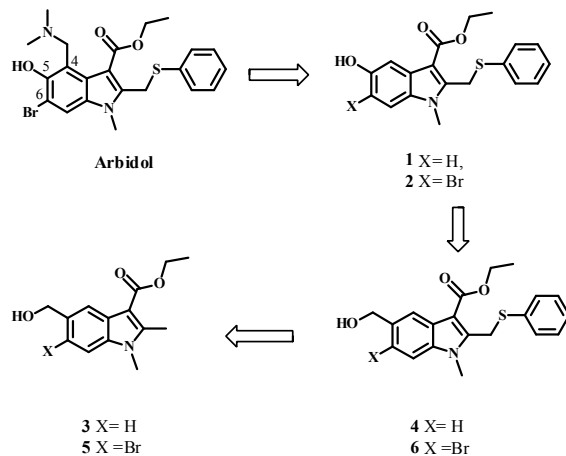
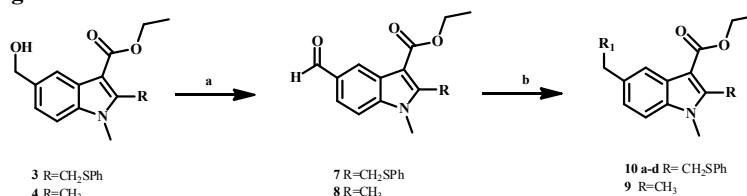


Figure 1: Structural modifications on Arbidol for selection of our lead compound

2.1 Synthesis of analogues of arbidol



Scheme 1. Reagents and conditions: a) Pyridinium dichromate, CH_2Cl_2 , 1h, r.t.; b) Aliphatic amines, Acetic acid, $\text{NaBH}(\text{OAc})_3$, dry 1,2 dichloroethane, r.t.

Indole derivatives **3** and **4** oxidised with pyridinium dichromate in CH_2Cl_2 to obtain aldehydes **7** and **8** respectively. Aldehydes **7** and **8** are treated with amines and then with $\text{NaBH}(\text{OAc})_3$. This reductive amination i. e. conversion of aldehydes **7** and **8** into amines **10** and **9**, was carried out in dry 1,2 dichloroethane, in the presence of acetic acid, using $\text{NaBH}(\text{OAc})_3$ reducing agent. The analytical data is in agreement with the literature.¹²

2.2 Biological activity of arbidol analogues 9 and 10a-d

The effect on the replication of Chikungunya virus of Arbidol and its analogues were tested *in verocells*. The cytotoxicity assay was performed in the same cell line. The results are reported in Table 1. Most of the compounds didn't show any inhibitory effect on Chikungunya virus replication. In the alcohol series, no activity was found and only compound **3** showed $\text{EC}_{50} = 56 \text{ mg/mL}$, higher than our lead compound. Four compounds in the amine series (**10 a-d**) showed an interesting activity when compared to Arbidol, but unfortunately, they have very high cytotoxicity in the host cells. It is worthy to note that all these compounds possess a thiophenol moiety in position 2 of the indole nucleus, and when we compared the same compounds in which thiophenol moiety was removed (for example compounds **10 c** and **9**) it was possible to note absence of effect and cytotoxicity.

Table 1: Anti-CHIKV activities and cytotoxicity of synthesized compounds in vero cells

Compound	X	n	R	R ₁	CC ₅₀	EC ₅₀	SI
Arbidol	-	-	-	-	83	18.4	4.52
9	H	1	-CH ₃	-N(CH ₃)CH ₂ CH ₂ CH ₂ N(CH ₂ CH ₃) ₂	ND	>258	ND
10a	H	1	-CH ₂ SPh	-N(CH ₂ CH ₃) ₂	21.6	4.16	5.18
10b	H	1	-CH ₂ SPh	-N(CH ₃)CH ₂ CH ₂ CH ₂ N(CH ₂ CH ₃) ₂	2.93	<1.61	1.81
10c	H	1	-CH ₂ SPh	-pyrrolidine	16.6	5.41	3.07
10d	H	1	-CH ₂ SPh	-N-tert-butylloxycarbonyl piperazine	15.5	4.3	3.61

CC_{50} (mg/mL) = 50% Cytostatic/Cytotoxic Concentration (Concentration at which 50% adverse effect is observed in the host cells). EC_{50} (mg/mL) = Effective Concentration (Concentration at which 50% inhibition of virus replication is observed). SI = Safety index (CC_{50}/EC_{50}).

This suggested that the introduction of thiophenol moiety is essential for the activity on virus replication but also responsible of cytotoxicity in the host cells. Thus, structural modifications are desirable to converting them in sulfoxides as previously reported.¹¹

III. EXPERIMENTAL

The experimental procedure is used as it is from literature.¹²

Ethyl-5-[[[4-(diethylamino)-1-methyl-butyl]amino]methyl]-1-methyl-2-methyl-1H-indole-3-carboxylate (9)

Elution with Hexane/Ethyl acetate 7/3. Yellow oil; Yield: (0.080 g) 96%. ¹H NMR (300 MHz, CDCl₃): δ 7.99 (s, 1H), 7.21 (s, 2H), 4.35 (q, *J* = 7.2 Hz, 2H), 3.89 (app q, *J* = 11.7 Hz, 2H, AB system), 2.70 (s, 3H), 3.62 (s, 3H), 2.36-2.48 (m, *J* = 7.2 Hz, 7H), 1.36-1.50 (m, 5H), 1.11 (d, *J* = 5 Hz, 3H), 0.99 (t, *J* = 7.4 Hz, 6H). ¹³C NMR (75 MHz, CDCl₃): δ 166.2, 140.3, 136.2, 124.4, 123.1, 120.7, 118.7, 111.3, 103.2, 62.8, 61.1, 54.2, 51.8, 49.1, 43.3, 35.6, 25.2, 14.1, 13.4, 5.3. MS (ESI): *m/z* = 374.87 [M + H⁺]. Anal. calcd for C₂₂H₃₅N₃O₂: C, 70.74; H, 9.44; N, 11.25, Found: C, 70.93; H, 9.77; N, 10.97.

Ethyl-5-(diethylaminomethyl)-1-methyl-2-(phenylsulfanylmethyl)-1H-indole-3-carboxylate(10a)

Purified using Ethyl acetate/MeOH/NH₃ 1/1/0.1. Dark oil; Yield: (83 mg) 90%. ¹H NMR (300 MHz, CDCl₃): δ 8.12 (s, 1H), 7.17-7.47 (m, 7H), 4.75 (s, 2H), 4.32 (q, *J* = 7.2 Hz, 2H), 3.76 (s, 2H), 3.68 (s, 3H), 2.60 (q, *J* = 7.2 Hz, 4H), 1.40 (t, *J* = 7.2 Hz, 3H), 1.12 (t, *J* = 7.2 Hz, 6H). ¹³C NMR (75 MHz, CDCl₃): δ 166.3, 141.9, 137.1, 135.3, 130.1, 127.6, 127.1, 126.1, 125.9, 120.8, 119.0, 111.3, 104.3, 61.3, 60.8, 49.8, 35.9, 23.3, 15.1, 13.8. MS (ESI): *m/z* = 411.63 [M + H⁺]. Anal. calcd for C₂₄H₃₀N₂O₂S: C, 70.21; H, 7.36; N, 6.82; S, 7.81, Found: C, 70.14; H, 7.23; N, 6.78; S, 7.73.

IV. CONCLUSION

In conclusion, a library of indole analogues of arbidol like derivatives have been designed, synthesized and used for testing their anti-viral activity on chikungunya virus (CHIKV).

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