Synthesis and molecular modeling study of novel pyrrole Schiff Bases as anti-HSV-1 agents

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Abstract: A series of novel pyrrole Schiff bases were synthesized by reaction of 2-amino-1,5-diaryl pyrrole-3-carbonitrile **1a-h** with different aromatic aldehydes using P_2O_5 as a catalyst to obtain **2a-p**, which were evaluated against herpes simplex virus type1 (HSV-1). The compounds **2d**, **2h**, **2m**, and **2n** were found to reduce the virus yield by 94-99 %, while compounds **2g**, **2k** and **2o** showed moderate activity (65-70%) compared to ACV (96%). The rest of the compounds were found to be inactive against HSV-1. Molecular modeling studies were carried out through docking the compounds in its most stable conformation into the active site of HSV-1 TK. The study revealed that the best fitted conformer was **2n** with a higher docking score (-7.32) and a better binding mode than ACV. The O-atom of the OCH_3 group formed two H-bond interactions with Arg^{176} and Tyr^{101}, hydrogen bonds were also formed with Arg^{163} and Tyr^{132} all of which are crucial amino acid residues for the enzyme activity, in addition to a H-bond interaction between the N^2-pyrazole and Gly^{59}. It could be suggested that the van der Waal interactions demonstrated by 1, 5-diaryl pyrrole located the molecule in close proximity to the active site Arg^{176}, Tyr^{101}, Arg^{163} and Tyr^{132}.

[Khalid M. H. Hilmy, Dalia H. Soliman, Esmat B. A. Shahin and Rakia Abd Alhameed. **Synthesis and molecular modeling study of novel pyrrole Schiff Bases as anti-HSV-1agents.** Life Sci J 2012;9(2):736-745] (ISSN:1097-8135). http://www.lifesciencesite.com. 109

Keywords: Pyrrole Schiff bases/HSV-1/HSV-Thymidine Kinase/Molecular modeling studies.

1. Introduction

Herpes simplex virus (HSV) causes herpes labialis, herpes keratitis, genetic herpes and life-threatening herpes encephalitis. HSV infections are more severe in immunocompromised patients, which are characterized by chronic and extensive lesions of the mucous membranes [1]. Most therapies directed against HSV infections are nucleotides, nucleosides or pyrophosphate analogues, such as acyclovir (ACV), valacyclovir, penciclovir and famciclovir. After uptake by virus-infected cells, these drugs are phosphorylated by virus-encoded thymidine kinase (TK), compete with the nucleotides to inhibit the viral DNA polymerase and subsequently cause the termination of growing viral DNA chains [2]. Although these drugs are effective in the treatment of many acute infections, the intensive use of these drugs has led to the emergence of resistant viral strains, mainly in immunocompromised patients. Inspite of the substantial advance in HSV therapy through the introduction of ACV, this anti-HSV compound and most of the other compounds under pharmaceutical development are substrate analogs of thymidine kinase [3]. It is important to note that TK is not a direct target of antiviral therapy because it is not required for virus replication; however viral TK activity may be required for reactivation of the virus from latency in the nerve cells [4]. Since antiviral drug resistance has become an issue of increasing clinical importance, the need for structurally unrelated agents which incorporate novel mechanisms of viral inhibition is apparent. Therefore, search for new non-nucleoside compounds as anti-HSV could be useful TK inhibitors. Pyrrolo[2,3-*d*]pyrimidines are purine analogs that display remarkable biological activities such as ant-inflammatory [5], anticancer[6-10], antimicrobial [11], antibiotics containing this moiety [12], antiasthmatic [13] and antiviral [14-15]. In addition, Tubercidine and Sangivamycin are pyrrolo[2,3-*d*]pyrimidine nucleoside antibiotics isolated from *Streptomyces* species that inhibit HSV replication [16]. Many bioactive natural products and synthetic drugs contain a pyrrole moiety as their key skeleton [17,18], subsequently highly substituted pyrroles has been one of the major targets in synthetic chemistry. Virtually, they were found to possess a wide variety of biological activities such as antibacterial[19], anticancer [20-23], antifungal [24], anti-inflammatory [25-28] and anti-oxidants [29].Many studies have reported on the antiviral activity of pyrroles [30-33], interestingly it was established that Congocidine and Distamycin are pyrroleamidine antibiotics isolated from *Streptomyces chromagens* that inhibits the multiplication of DNA virus HSV [34,35].Moreover, Schiff bases derived from pyrrole were effective antiviral agents [35].Schiff bases also deserve great interest due to their biological properties [36].The

above mentioned facts encouraged us to undertake the synthesis of some new Schiff bases of 2-amino-3-cyano-1,5-disubstitutedpyrrole to be evaluated as anti HSV-1.

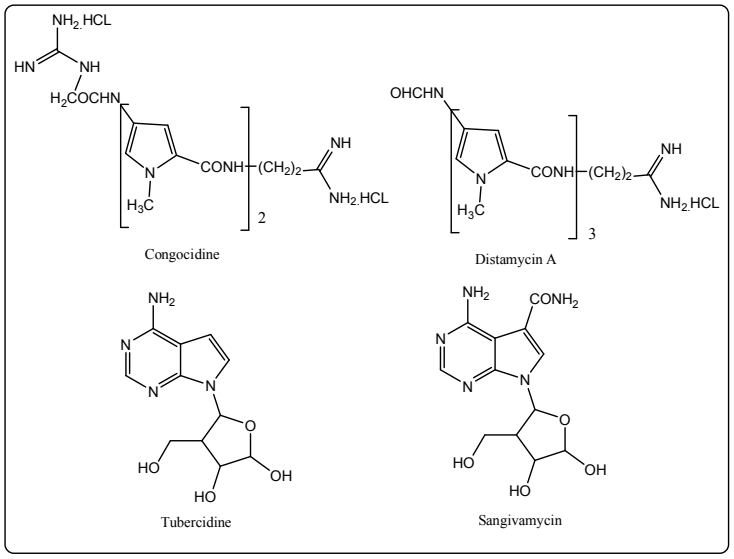
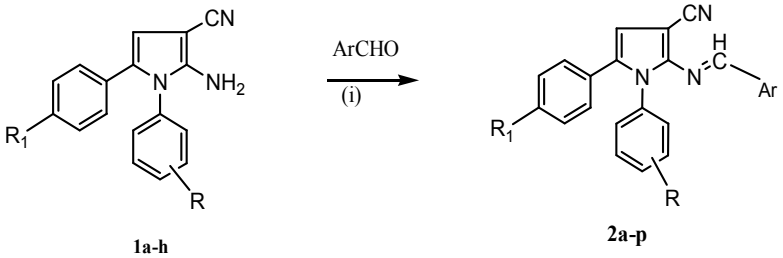


Figure 1. Structures of selective natural pyrrole and pyrrolopyrimidine lead compounds used as anti HSV-1.

2. Results and Discussion

The synthetic route utilized for the synthesis of the target compounds is outlined in Scheme 1. Condensation of 2-amino-3-cyano-1,5-diarylpyrroles 1a-h [37] with various aryl aldehydes in the presence of a catalytic amount of phosphorous pentoxide (P_2O_5)for 2-12 hrs yielded the Schiff bases 2a-p. The structures of the new compounds 2-arylideneamino-1,5-diaryl-1H-pyrrole-3 carbonitriles2a-p were confirmed by spectral data. The main feature characterizing their IR spectra is the presence of CN band at 2202-2212 cm^{-1}, the disappearance of the band corresponding to NH_2 group and the detection of a strong C=N stretching band at 1580-1645 cm^{-1} evidenced the formation of the Schiff base. Moreover, ^{1}H-NMR of the synthesized compounds revealed a singlet at around δ6.6 attributed to the pyrrole hydrogen, another singlet characterized the ^{1}H-NMR spectra at approximately δ 9.1 corresponding to the imine hydrogen. The ^{13}C{1H} NMR spectrum of 2j in DMSO-d_6 showed downfield signals at δ 162.7 ppm (C_2-naphthyl), 159.8 ppm (C-imine), 157.8 ppm (C_4'-phenyl) as well as a signal to up field at δ 55.5 ppm (OCH_3). It is worth mentioning here that, the use of the strong dehydrating agent phosphorous pentoxide (P_2O_5) in catalytic amount allowed the reaction to proceed forward in much less time (TLC monitored) and significantly higher yields. Different structural aldehydes were used to conduct a comparative study on the time of the reaction as well as its yield. The reaction of O-vanillin and salisaldehyde took place easily with good yield especially when pyrrole derivatives with substitution on two phenyl group were in para position. The reactions of cinnamaldehyde were faster and gave better yield than the pyrazole-4-carbaldehyde derivatives.



Compound No.	R	R_1
1a	4-Cl	H
b	3-CF_3	H
c	4-OCH_3	4-Cl
d	H	4-Br
e	4-CH_3	H
f	H	H
g	4-Br	H
h	4-OCH_3	H

(i)Absolute ethanol, P_2O_5, reflux.

Scheme1. Synthesis of Schiff bases of 2-amino-3-cyano-1,5-diaryl-pyrroles.

3. Antiviral Screening

The newly synthesized compounds **2a-p** were evaluated for their *in vitro* antiviral activity against herpes simplex virus type 1. The antiviral screening was performed using the plaque reduction assay against HSV-1 in vero cell; ACV was used as the standard drug showing 96% reduction in the number of virus plaques. Table 1 summarizes the anti HSV-1 activity of these compounds with respect to Acyclovir. Compounds **2d**, **2h**, **2m**, and **2n** reduced the virus yields by 99-94 %. Both **2d** and **2n**proved to be the most active among the tested compounds and even more than ACV by reducing the number of HSV-1 plaques by 99% and 97% respectively. The antiviral activity of compounds **2h** and **2m** was comparable to the standard ACV, 96% and 94% respectively. Compounds **2g**, **2k** and **2o** showed moderate activity (65-70%) compared to ACV (96%). The rest of the compounds were found to be inactive against HSV-1.Extensive SAR exploration of this novel series showed that the 5-pyrrole position of the most active compounds **2d** and **2n** was occupied by a 4-methoxyphenyl, the highest activity was achieved by compound **2d** which also had a 4-methoxybenzylideneamino in position 2 of the pyrrole ring. However, a total loss in the activity was conferred when the 4-methoxybenzylideneamino was replaced by 2-hydroxy-4-methoxybenzylideneamino. The high activity of **2n** could be attributed to the combination of the 4-methoxyphenyl and the pyrazole ring which itself has reported antiviral activity [38].The Schiff base derived from cinnamaldehyde **2g** expressed moderate antiviral activity thus, we aimed to enhance the activity by replacing the 5-phenylpyrrole with a 4-methoxyphenl counterpart, unfortunately this resulted in an inactive compound **2i**, while 5-(4-bromophenyl) pyrrole**2h** showed the same activity as ACV. The moderate activity of compounds **2k** and **2o** could be attributed to the reported antiviral activity of quinoline and pyrazole [38,39]. Bulky rigid structures as **2j** and **2l** were devoid of antiviral activity. The results indicate that there is no main substituent on the pyrrole that is responsible for the activity, instead all those substituents work together to bring up activity and help position the compound in the best fit into the enzyme.

The experimental concentrations of the 1,5-diaryl-2-(ylideneamino)-1*H*-pyrrole-3-carbonitriles (**2a-p**) were 50 µM and ACV concentration was 10 µM.

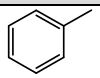
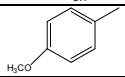
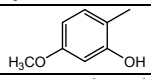
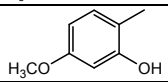
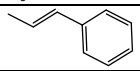
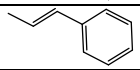
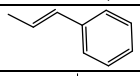
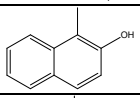
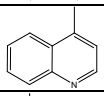
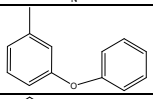
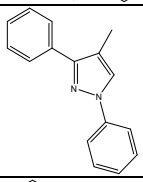
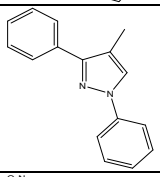
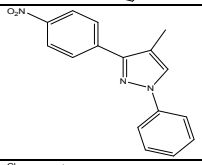
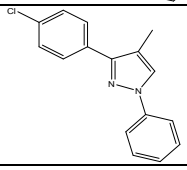
a) % per cent of reduction = [1-(t/c)] x 100
b) NA: Non active
c) ACV: acyclovir has been included for comparison purposes

1. Docking Studies

Thymidine kinase acts catalytically to phosphorylate thymidine, getting it ready for further phosphorylation and eventual incorporation into DNA [40].Thus, the research into the binding capabilities of TK and other ligands can serve to develop more effective HSV treatment that would act as inhibitors of this enzyme rather than substrates. In the present study, the ligand-receptor interactions of compounds **2d, 2h, 2m** and **2n** with herpes simplex virus type-1 thymidine kinase were investigated by performing docking studies using Molecular Operating Environment (MOE)version 2008.10[41].The crystal structure of herpes simplex virus type-1 thymidine kinase in complex with acyclovir (PDB code 1KI5) [42] was retrieved from the Protein Data Bank [43] and the accuracy of MOE docking protocol was validated and confirmed by docking the co-crystallized acyclovir inside the active site of thymidine kinase where the docked acyclovir showed 1.7841 Å root-mean-squared deviation fig.2.The compounds were docked in their most stable conformation in the active site of HSV-1 TK and subjected to energy minimization. The docking scores are presented in Table 2.

The docking interaction of compound **2d** with the active site of thymidine kinase is represented in figure 3. It was observed that the oxygen of the two methoxy groups formed H-bond acceptor interactions with Lys^{62} (2.8 Å) and Tyr^{132} (3.2 Å) two of the amino acids in the active site of the enzyme [40, 42, 44, 45], Thr^{63} (2.7, 3.6 Å) in addition to H-bond acceptor interaction between the cyano nitrogen and Arg222 (2.8 Å). Hydrophobic interactions between pyrrole and His^{58} as well as the phenyl ring with Arg^{222} and Lys^{62} appear to constrain the molecule in close proximity with the amino acids forming the forementioned hydrogen bonding. Moreover, compound **2d** formed van der Waals interactions with non polar atoms of (Trp^{88}, Ile^{100}, Tyr^{101}, Gln^{125}, Met^{128}, Tyr^{132}, Asp^{162}, Arg^{163}, Ala^{168}, Tyr^{172}, Arg^{176} and Arg^{222}) similar to those reported for the ligands deoxythymidine (dT) and ACV [40,42,45], in addition to hydrophobic interactions with (Glu^{83}, Glu^{225}, Gly^{59}, Gly^{61}, Ile^{97}).

Table 1. The Anti-HSV-1 activity of the 1,5-diaryl-2-(ylideneamino)-1*H*-pyrrole-3-carbonitriles (2a-p)

Compound No.	R	R_1	Ar	Cytotoxicity of compound	% Reduction in cytopathic effect of HSV-1[a]
2a	4-Cl	H		10^{-2}	NA [b]
2b	4-Cl	H		10^{-2}	NA
2c	3-CF_3	H		10^{-2}	NA
2d	4-OCH_3	4-Cl		10^{-2}	99
2e	H	4-Br		10^{-2}	NA
2f	4-CH_3	H		10^{-2}	NA
2g	H	H		10^{-2}	65
2h	4-Br	H		10^{-2}	96
2i	4-OCH_3	4-Cl		10^{-2}	NA
2j	4-OCH_3	4-Cl		10^{-2}	NA
2k	H	4-Br		10^{-2}	67
2l	H	H		10^{-2}	NA
2m	H	H		10^{-2}	94
2n	4-OCH_3	H		10^{-2}	97
2o	H	H		10^{-2}	70
2p	4-Br	H		10^{-2}	NA
ACV[c]	-	-	-	10^{-2}	96

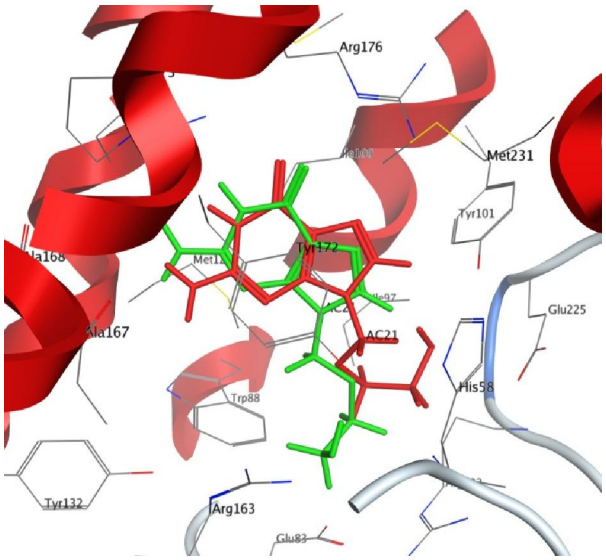


Figure 2. Comparison between the co-crystallized acyclovir (red) and Docked acyclovir (green).

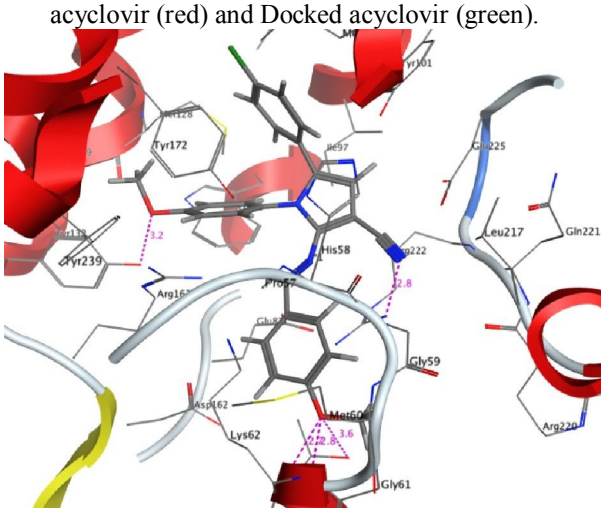


Figure 3. Interaction of compounds **2d** with thymidine kinase.

Table 2. **Docking scores of the acyclovir, 2d, 2h, 2m and 2n.**

Compound	Docking Score
Acyclovir	-12.74
2d	-11.30
2h	-11.44
2m	-8.98
2n	-7.32

The binding mode observed for compound **2n** shows that it binds within the deoxythymidine binding pocket with a docking score better than ACV (-7.32), the oxygen of the OCH_3 forming two hydrogen bonds, one with Arg^{176}(2.8Å), a crucial residue in the TK binding site, and another one with Tyr^{101} (2.8 Å) that resembles the bond made by the 3' O of dT [42,46]. The imine nitrogen establishes a hydrogen bond with the residue Arg^{163}(2.6Å), which is responsible for making hydrogen bond interaction with the 5' O of dT [40, 46]. Additionally the cyano nitrogen forms a hydrogen bond with Tyr^{132}(3.2 Å) and another one is observed between theN^2-pyrazoleand with Gly^{59} (3.0Å). Further stabilization is obtained by hydrophobic interactions with His^{58} and by van der Waal interactions between (Tyr^{132}, Arg^{163}, Ala^{168}, Trp^{88}, Met^{231}, Met^{128},Tyr^{172}) as reported [40,42,45], other hydrophobic interactions were also observed withGly^{59}, Gly^{61}, Lys^{62}, Thr^{63}, Thr^{64}, Glu^{83}, Ile^{97}, Tyr^{101}, Gln^{125}, Asp^{162}, Arg^{176}, Arg^{220}andGlu^{225} (figure 4).It is worth mentioning here, that Arg^{222} formed strong hydrophobic interactions with 1,3-diphenyl pyrazole moiety that could be responsible for bringing the molecule into close proximity with the crucial active sites of the enzyme.

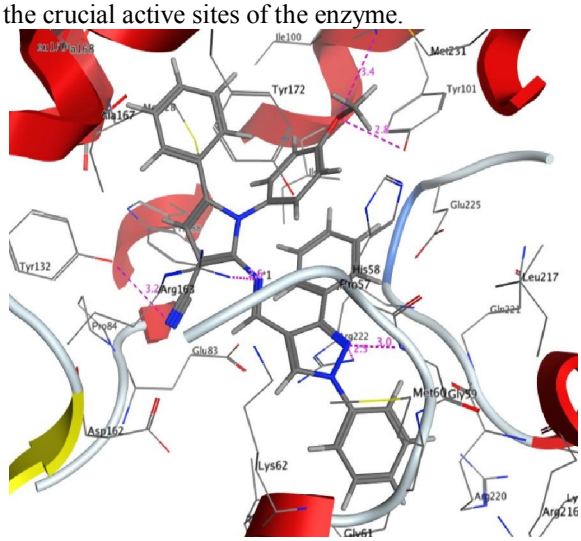


Figure 4. Interaction of compounds **2n** with thymidine kinase

Given that the pyrrole derivative **2n** presentation is similar to that of dT and Acyclovir, and that ACV is considered a weak substrate [47], the potential phosphate-binding function (the hydroxyl group) was replaced by a methoxy group. It would appear that its inhibitory properties arise from the much greater binding enthalpy that the van der Waals interactions of the additional hydrophobic moieties give rise to. The high affinity, arising, no doubt, from these interactions, may restrict the flexibility of the complex to allow phosphorylation of the ligand [42].In case of compound **2h** (supplementary data), only hydrophobic interactions were observed with His^{58}, Gly^{59}, Lys^{62}, Thr^{63}, Glu^{83}, Trp^{88}, Ile^{97}, Ile^{100}, Tyr^{101}, Met^{128}, Tyr^{132}, Asp^{162}, Arg^{163}, Ala^{167}, Ala^{168}, Tyr^{172}, Arg^{176}, Arg^{222} and Glu^{225}. Similarly, compound **2m** showed only hydrophobic interactions with His^{58}, Gly^{59}, Lys^{62}, Thr^{63}, Glu^{83}, Trp^{88}, Ile^{97}, Ile^{100}, Tyr^{101}, Gln^{125}, Met^{128}, Tyr^{132}, Asp^{162}, Arg^{163}, Ala^{167}, Ala^{168}, Tyr^{172}, Arg^{176}, Arg^{222} and Glu^{225}(supplementary data). Compounds **2h** and **2m**

although possessed a significant antiviral activity and their docking scores were comparable to ACV yet their docking into TK did not show any hydrogen bonding interactions in the binding site of the enzyme, suggesting that the compounds may have another mechanism for their antiherpetic activity.

Conclusion

In this article, a series of new pyrrole Schiff bases**2a-p** has been synthesized. The new pyrrole Schiff bases **2a-p** were tested for anti HSV-1 activity in vero cell. The tested compounds **2d** and **2n** demonstrated potent antiviral activity against HSV-1 more than the standard drug. Also, the compounds**2h** and **2m** exhibited antiviral activity comparable to the standard drug. However, the rest of the pyrrole Schiff bases didn't have such an influence on activity. Molecular modeling studies showed that the binding mode of compound **2n**, in spite of its size difference, was very similar to the previously reported ACV and dT. Thus, **2n** could be a potential ligand to target /inhibit TK of herpes simplex virus.

2. Experimental

2.1. General

Melting points (^{o}C uncorrected) were recorded with a Gallenkamp apparatus (Weiss-Gallenkamp, London, UK). The IR spectra were recorded on KBr pellets on a Jasco FT/IR 460 plus (Japan). ^{1}HNMR and ^{13}CNMR spectra were recorded on Varian Gemini spectrophotometer (200 MHz) in DMSO-d_6 or $CDCl_3$ as solvent, using tetramethyl-silane (TMS) as internal reference standard. The chemical shifts values are expressed in ppm (parts per million). Elemental analyses were performed by a Vario III CHN analyzer (Germany). All compounds were within ± 0.4% of the theoretical values. Mass spectra were performed on DI analysis Shimadzu QP-2010 plus mass spectrometer. All spectroscopic data and elemental analysis were made at the Micro analytical center, Cairo University, Egypt. The progress of the reaction and purity of the compounds were monitored by TLC analytical silica gel plates 60 F_{254} (E. Merck Germany) using the appropriate eluent. The chemical reagents used in synthesis were purchased from Fluka, Sigma and Aldrich.

2.2. Synthesis of compounds 1,2

2.2.1. General procedure for the synthesis of compounds 1a-h[37]

Derivatives of phenacylmalononitrile were refluxed with different anilines in ethanol in the presence of conc. HCl to give pyrrole derivatives **1a-h**.

2.2.2. General procedure for the synthesis of 2-(arylideneamino)-1,5-diaryl-*1H*-pyrrole-3 carbonitriles 2a-p

A mixture of **1a-h** (0.01mol) and various aromatic aldehydes (0.01mol) in ethanol (20 mL), in the presence of catalytically amount of P_2O_5 was refluxed for 2-12 hrs. The separated solid was filtered and recrystallized from ethanol.

2.2.2.1. 2-(Benzylideneamino)-1-(4-chlorophenyl)-5-phenyl-*1H*-pyrrole-3-carbonitrile (2a)

Yield 80%; m.p. 164-166 ^{0}C. IR (KBr) ν cm^{-1}: 2208 (CN); 1580 (C=N).^{1}HNMR spectrum ($CDCl_3$).δ ppm: 6.65 (s, 1H, $CH_{pyrrole}$); 7.11–7.75 (m, 14H, Ar-H); 9.12(s, 1H, -N=CH). MS m/z (%): 381(M^+, 19).Anal. Calcd for $C_{24}H_{16}ClN_3$ (381.86): C, 75.49; H, 4.22; N, 11.00.Found: C, 75.45; H, 4.25; N, 11.03.

2.2.2.2. 1-(4-Chlorophenyl)-2-(2-hydroxybenzylideneamino)-5-phenyl-*1H*-pyrrole-3-carbonitrile (2b)

Yield 81%; m.p. 176-178 ^{0}C. IR (KBr) νcm^{-1}: 2207 (CN); 3413 (OH); 1591 (C=N).^{1}HNMR spectrum ($CDCl_3$).δ ppm: 6.64 (s, 1H, $CH_{pyrrole}$), 7.10–7. 54 (m, 13H, Ar-H); 9.18 (s, 1H, -N=CH); 11.34(s, 1H, OH). MS m/z (%): 398 (M^+, 32); 400 (M^{+2}, 2).Anal. Calcd for$C_{24}H_{16}ClN_3O$ (397.86): C, 72.45; H, 4.05; N, 10.56. Found: C, 72.48; H, 4.08; N, 10.50.

2.2.2.3. 2-(2-Hydroxybenzylideneamino)-5-phenyl-1-3-(trifluoromethyl)-5-phenyl-*1H*-pyrrole-3-carbonitrile (2c)

Yield 78%; m.p. 178-180 ^{0}C. IR (KBr) νcm^{-1}: 2210 (CN); 3410 (OH); 1599 (C=N).^{1}HNMR spectrum ($CDCl_3$).δ ppm: 6.67 (s, 1H, $CH_{pyrrole}$); 7.11–7. 61 (m, 13H, Ar-H); 8.92 (s, 1H, -N=CH); 11. 29 (s, 1H, OH). MS m/z (%): 431 (M^+, 100).Anal. Calcd for $C_{25}H_{16}F_3N_3O$ (431.41): C, 69.60; H, 3.74; N, 9.74. Found: C, 69.57; H, 3.76; N, 9.72.

2.2.2.4. 5-(4-Chlorophenyl)-1-(4-methoxyphenyl)-2-(4-methoxybenzylideneamino)-1H-pyrrole-3-carbonitrile (2d)

Yield 83%; m.p. 220-222 ^{0}C. IR (KBr) νcm^{-1}: 2206 (CN); 1620 (C=N). ^{1}HNMR spectrum ($CDCl_3$).δ ppm: 3.92(s, 6H, $2OCH_3$); 6.68(s, 1H, $CH_{pyrrole}$); 7.00–7. 54 (m, 12H, Ar-H); 9.11(s, 1H, -N=CH). MS m/z (%): 441 (M^+, 32); 443 (M^{+2}, 21).Anal. Calcd for $C_{26}H_{20}ClN_3O_2$ (441.91): C, 70.67; H, 4.56; N, 9.51.Found: C, 70.70; H, 4.59; N, 9.48.

2.2.2.5. 5-(4-Bromophenyl)-2-(2-hydroxy-4-methoxybenzylideneamino)-1-phenyl-1H-pyrrole-3-carbonitrile (2e)

Yield 80 %; m.p. 218-220 ^{0}C. IR (KBr) νcm^{-1}: 2210 (CN); 3412(OH); 1643 (C=N).^{1}HNMR spectrum ($CDCl_3$).δ ppm: 3.89 (s,3H, OCH_3); 6.70 (s, 1H, $CH_{pyrrole}$); 7.00–7. 52 (m, 12H, Ar-H); 9.33(s, 1H, -N=CH); 11. 27(s, 1H, OH).MS m/z (%): 471 (M^+, 26); 473 (M^{+2}, 15).Anal. Calcd for $C_{25}H_{18}BrN_3O_2$ (472.33): C, 63.57; H, 3.84; N, 8.90. Found: C, 63.54; H, 3.87; N, 8.88.

2.2.2.6. **2-(2-Hydroxy-4-methoxybenzylideneamino)-5-phenyl-1-(p-tolyl)-*1H*-pyrrole-3-carbonitrile (2f)**

Yield 79 %; m.p. 230-232 ^{0}C. IR (KBr) νcm^{-1}: 2212 (CN); 3408 (OH); 1592 (C=N).^{1}HNMR spectrum (DMSO-d_6).δ ppm:2.37 (s, 3H, CH_3); 3.82 (s, 3H, OCH_3); 6.69 (s, 1H,$CH_{pyrrole}$); 7.00–7.26 (m, 12H, Ar-H); 9.26 (s, 1H, -C=NH); 11.29 (s,1H,OH). MS m/z (%): 407 (M^+, 100).Anal. Calcd for $C_{26}H_{21}N_3O_2$ (407.46): C, 76.64; H, 5.19; N, 10.31.Found: C, 76.63; H, 5.16; N, 10.29.

2.2.2.7. **1,5-Diphenyl-2-(3-phenylallylideneamino)-1H-pyrrole-3-carbonitrile (2g)**

Yield 75%; m.p. 184-186 ^{0}C. IR (KBr) νcm^{-1}: 2210 (CN); 1609 (C=N). ^{1}HNMR spectrum (DMSO-d_6).δ ppm: 6.65 (s, 1H, $CH_{pyrrole}$); 7.07(t, 1H, CH=CH); 7.10–7.41 (m, 15H, Ar-H); 7.70-7.72 (d, 1H, CH=CH- Ph); 8.77-8.83 (d, 1H, -N=CH). MS m/z (%): 373 (M^+, 100).Anal. Calcd for $C_{26}H_{19}N_3$ (373.45): C, 83.62; H, 5.13; N, 11.25. Found: C, 83.65; H, 5.10; N, 11.28.

2.2.2.8. **1-(4-Bromophenyl)-5-pheny-2-(3-phenylallylideneamino)-*1H*-pyrrole-3-carbonitrile (2h)**

Yield 74 %; m.p. 208-210 ^{0}C. IR (KBr) νcm^{-1}: 2210 (CN); 1622 (C=N). ^{1}HNMR spectrum (DMSO-d_6).δ ppm: 6.69 (s, 1H, $CH_{pyrrole}$); 7.08 (t, 1H, CH=CH); 7.11–7.54 (m, 14H, Ar-H); 7.70-7.72 (d, 1H, CH=CH-Ph); 8.75-8.80 (d, 1H, HC=N).MS m/z (%): 452 (M^+, 37); 454 (M^{+2}, 8).Anal. Calcd for $C_{26}H_{18}BrN_3$ (452.35): C, 69.04; H, 4.01; N, 9.29.Found: C, 69.00; H, 3.99; N, 9.27.

2.2.2.9. **5-(4-Chlorophenyl)-1-(4-methoxyphenyl)-2-(3-phenylallylideneamino)-*1H*-pyrrole-3-carbonitrile (2i)**

Yield 80 %; m.p. 252-254 ^{0}C. IR (KBr) νcm^{-1}: 2210 (CN); 1588 (C=N). ^{1}HNMR spectrum (DMSO-d_6).δ ppm: 3.80 (s, 3H, OCH_3); 6.91 (s, 1H, $CH_{pyrrole}$); 7.07(t, 1H, CH=CH); 7.11–7.40 (m, 13H, Ar-H); 7.70-7.72 (d, 1H, CH=CH- Ph); 8.61-8.70 (d, 1H, HC=N). MS m/z (%): 437 (M^+, 35), 439 (M^{+2}, 12).Anal. Calcd for $C_{27}H_{20}ClN_3O$ (437.92): C, 74.05; H, 4.60; N, 9.60.Found: C, 74.04; H, 4.63; N, 9.57.

2.2.2.10. **5-(4-Chlorophenyl)-2-[(2-hydroxynaphthalen-1-yl)methyleneamino]-1-(4-methoxyphenyl)-*1H*-pyrrole-3-carbonitrile (2j)**

Yield 79%; m.p. 237-239 ^{0}C. IR (KBr) νcm^{-1}: 2208 (CN); 3396 (OH); 1614 (C=N). ^{1}HNMR spectrum (DMSO-d_6).δ ppm: 3.83 (s, 1H, OCH_3); 6.72 (s, 1H, $CH_{pyrrole}$); 7.11-8.28 (m, 14H, Ar H); 10.11 (s, 1H, -N=CH); 12.93 (s, 1H, OH). ^{13}CNMR ($CDCl_3$) δ ppm: 55.53 (OCH_3); 111.94 (C_1-naphthyl); 114.84 (CN); 119.10, 120.00, 124.11, 128.01, 128.68, 129.31 (C-arom); 131.05 (N-C_1-phenyl); 132.87(C_4-pyrrole); 135.98(C-Cl); 157.89 (C-OCH_3); 159.86 (C=N); 162.73 (HO-C_2-naphthyl). MS m/z (%): 477 (M^+, 100); 479(M^{+2}, 33).Anal. Calcd for $C_{29}H_{20}ClN_3O_2$ (477.94): C, 72.88; H, 4.22; N, 8.79.Found: C, 72.86; H, 4.23; N, 8.81.

2.2.2.11. **5-(4-Bromophenyl)-1-phenyl-2-[(quinolin-4-yl)methyleneamino]-*1H*-pyrrole-3-carbonitrile (2k)**

Yield 72 %; m.p. 254-256 ^{0}C. IR (KBr) νcm^{-1}: 2211 (CN); 1609 (C=N). ^{1}HNMR spectrum ($CDCl_3$).δ ppm: 6.69 (s, 1H, $CH_{pyrrole}$); 7.22–8.10 (m, 15H, Ar-H); 8.34 (s, 1H, -N=CH); 9. 32 (s, 1H, N=$CH_{quinoline}$). MS m/z (%): 476 (M^+, 16); 478 (M^{+2}, 21).Anal. Calcd for $C_{27}H_{17}BrN_4$ (477.35): C, 67.93; H, 3.59; N, 11.74.Found: C, 67.90; H, 3.62; N, 11.70.

2.2.2.12. **1,5-Diphenyl-2-(3-phenoxybenzylideneamino)-*1H*-pyrrole-3-carbonitrile (2l)**

Yield 70 %; m.p. 186-188 ^{0}C. IR (KBr) νcm^{-1}: 2208 (C≡N); 1607 (C=N). ^{1}HNMR spectrum ($CDCl_3$).δ ppm: 6.70 (s, 1H, $CH_{pyrrole}$); 7.01–7. 74 (m, 19H, Ar-H); 8.32 (s, 1 H, - N=CH). MS m/z (%): 439 (M^+, 79).Anal. Calcd for $C_{30}H_{21}N_3O$ (439.51): C, 81.98; H, 4.82; N, 9.56.Found: C, 81.97; H, 4.80; N, 9.63.

2.2.2.13. **2-[(1,3-Diphenyl-1H-pyrazol-4-yl)methyleneamino]-1,5-diphenyl-*1H*-pyrrole-3-carbonitrile (2m)**

Yield 74 %; m.p. 204-206 ^{0}C. IR (KBr) νcm^{-1}: 2209 (CN); 1644 (C=N). ^{1}HNMR spectrum (DMSO-d_6).δ ppm: 6.66 (s, 1H, $CH_{pyrrole}$); 7.12-7.97 (m, 20H, Ar-H); 8.63 (s, 1H, -N=CH); 9.17 (s, 1H, $CH_{pyrazole}$). MS m/z (%): 489 (M^+, 91).Anal. Calcd for $C_{33}H_{23}N_5$ (489.57): C, 80.96; H, 4.74; N, 14.31.Found: C, 80.93; H, 4.70; N, 14.28.

2.2.2.14. **2-[(1,3-Diphenyl-1H-pyrazol-4-yl)methyleneamino]-1-(4-methoxyphenyl)-5-phenyl-*1H*-pyrrole-3-carbonitrile (2n)**

Yield 75%; m.p. 202-204 ^{0}C. IR (KBr) νcm^{-1}: 2209 (CN); 1599 (C=N). ^{1}HNMR spectrum (DMSO-d_6).δ ppm: 3.82 (s, 3H, OCH_3); 6.71 (s, 1H, $CH_{pyrrole}$); 7.11-7.67 (m, 19H, Ar-H); 8.42 (s, 1H, CH =N); 9.15 (s, 1H, $CH_{pyrazole}$). MS m/z (%): 519 (M^+, 30).Anal. Calcd for $C_{34}H_{25}N_5O$ (519.60): C, 78.59; H, 4.85; N, 13.48.Found: C, 78.56; H, 4.82; N, 13.44.

2.2.2.15. **1,5-Diphenyl-2-[(3-(4-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)methyleneamino]-*1H*-pyrrole-3-carbonitrile (2o)**

Yield 77%; m.p. 260-262 ^{0}C. IR (KBr) νcm^{-1}: 2209 (CN); 1645 (C=N). ^{1}HNMR spectrum (DMSO-d_6).δ ppm: 6.71 (s, 1H, $CH_{pyrrole}$); 7.11-7.97 (m, 19H, Ar-H); 8.23 (s, 1H, CH =N); 9.17 (s, 1H, $CH_{pyrazole}$). MS m/z (%): 534 (M^+, 100).Anal. Calcd for $C_{33}H_{22}N_6O_2$ (534.57): C, 74.14; H, 4.15; N, 15.72.Found: C, 74.16; H, 4.14; N, 15.69.

2.2.2.16. **1-(4-Bromophenyl)-2-[(3-(4-chlorophenyl)-1-phenyl-*1H*-pyrazol-4-yl)methyleneamino]-5-phenyl-1H-pyrrole-3-carbonitrile (2p)**

Yield 73%; m.p. 210-212 ^{0}C. IR (KBr) νcm^{-1}: 2209 (CN); 1602 (C=N).^{1}HNMR spectrum (DMSO-d_6).δ ppm: 6.81 (s, 1H, $CH_{pyrrole}$); 7.12-7.97 (m, 18H, Ar-H); 8.26 (s, 1H, -N=CH); 9.19 (s, 1H, $CH_{pyrazole}$). MS m/z (%): 603 (M^+, 27), 605 (M^{+2}, 8).Anal. Calcd for $C_{33}H_{21}BrClN_5$ (602.91): C, 65.74; H, 3.51; N, 11.62.Found: C, 65.71; H, 3.55; N, 11.65.

6.3. Antivirus activity

2.3. 1. Cells and virus

Herpes simplex Virus type-1 (HSV-1) was kindly supplied by The National Research Center, Dokki, Egypt. The virus was propagated in Vero cells (cells isolated from the African green monkey), which were commercially obtained from The Egyptian Organization of Serum and Vaccines. Minimal essential medium (MEM) was used as growth and maintenance medium for tissue culture with 10% or 2% fetal calf serum (FCS), respectively.

6.3.2. Titration of HSV-1 by plaque assay [48]

Cell suspension (3 ml) with the concentration 10^5 cells/ml GM was dispensed into each of the wells of 6-well plates (Falcon). Plates were incubated at 37^{o}C in a 0.5% CO_2 for 24–48 hrs to obtain a confluent sheet. Ten-fold dilution of the virus stock (10^{-1}–10^{-7}) was done as mentioned under titration of HSV by $TCID_{50}$ endpoint. Then growth medium was aspirated from the wells and 0.2 mL/well of each virus dilution was added in duplicate manner. For each plate, two wells were left uninoculated as cell control, each contained 0.2 mL MEM only; incubation at 37^{o}C in a 0.5% CO_2 incubator for one hour, to permit virus adsorption. Then 30 ml 1% agarose solution was melted at 48^{o}C in a water bath, and mixed with 2 x 30 mL MEM (supplied with 1% antibiotic solution and 2% FCS) in a ratio of 1:1 to prepare an overlay mixture. Then 3 ml of overlay mixture was added quickly to each well in the plate. After overlay solidification, plates were inverted and incubated at 37^{o}C in a 0.5% CO_2 incubator. Daily observation was carried out for early plaque detection; usually it takes 2–3 days for plaque detection. After plaques development, cells were fixed by flooding with 10% formalin solution for 1 h at room temperature. Agarose overlay was removed with forceps; cell monolayers were washed under tap water and stained with 10% crystal violet solution for 10 min. Plaques were recorded as clear unstained areas against a violet background of stained viable cells and counted either visually or using a stereomicroscope. The infectivity titer represented as number of plaque forming unit/mL (PFU/mL) of the stock virus suspension. It was calculated form the following equation: PFU/mL = No. of plaques x reciprocal of dilution x reciprocal of volume in mL.

6.3.3. Determination of the solvent Cytotoxicity

Growth medium was decanted from 96 well micro titer plate after a confluent sheet of cells was formed. Ten-fold serial dilutions of 2-(arylideneamino)-1,5-diaryl-1*H*-pyrrole-3-carbonitriles were made in MEM medium without FCS, starting from 5000 µm/mL till 10^{10} dilution, 0.2 ml of each dilution was tested in 3 different wells and leaving two wells/row as control receiving only maintenance medium. The plate was incubated in a CO_2 incubator at 37^{o}C and examined for up to 3 days. Cells were checked for any physical signs of toxicity, partial or complete loss of the monolayers, shrinkage, or cell granulation. Then, the non-toxic concentration of each substance was used in this study.

6.3.4. HSV-1 yield reduction assay

Vero cells were seeded into 96-well tissue culture plates at a concentration of 10,000 cells in 0.2 ML of minimal essential medium with Earle salts, MEM(E), supplemented with 10% FCS, and incubated at 37^{o}C in a humidified 3% CO_2 – 97% air atmosphere. After 24 hrs, plates were inverted over a waste vessel, medium was shaken out, and the plates were allowed to drain for 5 to 10 s on a sterile paper towel. Cultures were incubated with HSV-1 at a multiplicity of infection (MOI) of 5 PFU/cell in 0.2 mL of MEM (E) supplemented with 5% FCS, 100 U penicillin/mL, and 100 µg streptomycin sulfate/mL. Cultures were incubated at 37^{o}C for 2 hrs to permit virus adsorption. Virus inoculum's was replaced with 0.2 Ml of fresh medium and test compounds were added to the cultures.

The first row of 12 wells was left undisturbed and served as virus controls. Each well in the second row received an additional 0.1 mL of MEM (E) containing 5% FCS, antibiotics, and test compound at three times the desired final concentration. The contents of the 12 wells were mixed by repeated pipetting and then serially diluted 1:3 down the plate by repeated transfer and mixing of 0.1 mL of drug-containing medium. In this manner, six compounds could be tested in duplicate on a single plate with a concentrations range of nearly 1000-fold between the highest and the lowest dilutions (0.10 µM to 80 µM minuscule example). Plates were incubated at 37^{o}Cover night and then subjected to one cycle of freezing at –76^{o}C and thawing at 37^{o}C to disrupt the cells. Aliquots of 0.1 mL from each of the eight wells of a given row were transferred to the row of a fresh 96-well monolayers culture of Vero cells. Contents were mixed and serially diluted 1:3 across the remaining 11 rows of the second plate. Each row of the original primary plate was diluted across a separate plate in this manner. A culture was incubated

at 37°C for 2 h to permit virus adsorption and then the virus inoculum's was replaced with 0.2 mL of fresh medium. Cultures were incubated for 2 days, medium was removed, and the cell sheets were stained with0.1% crystal violet in 20% methanol. Plaques were counted under 20-fold magnification in the row of wells having the dilution which gave 5 to 20 plaques per well. Virus titers were calculated according to following formula:

titer (PFU/mL) = number of plaques x 5x 3^n; where n represents the n^{th} dilution of the virus used to infect the well in which plaques were counted.

6.4. Docking methodology

Docking studies were performed using Molecular Operating Environment (MOE) [41] version 2008.10 running on an Intel Core 2 Duo PC running Windows 7 as operating system. Crystal structure of herpes simplex virus type-1 thymidine kinase in complex with acyclovir (PDB code 1KI5) [43] was retrieved from the Protein Data Bank. From the PDB file of the complex, all the water molecules were removed and the missing hydrogens and partial charges were added using MOE. For ligands, 3D structures were constructed using the Builder module of MOE and optimized by energy minimization using MMFF94X force field. All docking calculations were carried out using the MOE Dock module. The parameters used were alpha triangle as placement methodology and London dG as scoring function with force field refinement. The highest scoring pose for each ligand was selected for further investigation. Ligand interactions were generated using the Ligand Interactions module in MOE.

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4/28/2012