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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Anti-HIV-1 screening of (2*E*)-3-(2-chloro-6-methyl/methoxyquinolin-3-yl)-1-(aryl)prop-2-en-1-ones

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Abstract Biological studies of two series of 38 quinolinyl chalcones (**4a–s** and **5a–s**) were investigated for their in vitro anti-HIV-1 and cytotoxic activities. Out of 38 compounds, seventeen compounds were observed as good anti-HIV-1 agents with EC_{50} values less than 20 μM. Compounds **4a**, **4l**, **4o**, **5e**, **5h**, **5l**, **5o**, and **5r** displayed potent anti-HIV-1 activity with EC_{50} values less than 5 μM. Among these, compound **5h** emerged as the best anti-HIV-1 agent (EC_{50} = 1.1 μM). Compounds **4d**, **4n**, **4q**, **4r**, **4s**, **5n**, and **5r** showed no toxicity in human lymphocytes. Bioassay results show that the type(s) and position(s) of the substituents seem to be critical for their cytotoxic and anti-HIV-1 activities. These results could be useful in the invention of new anti-HIV agents through structural modification.

Keywords Heterocyclic compounds · Quinolyl chalcones · Arylketone · Cytotoxicity · Anti-HIV-1 activity

This work is dedicated to the Late Professor Dr. Hamid Latif Siddiqui, Institute of Chemistry, University of the Punjab, Lahore, Pakistan.

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Introduction

Human immunodeficiency virus has been a continuous threat to human beings. It has resulted in the deaths of millions of people across the globe. Currently, highly active antiretroviral therapy (HAART) is being used for its treatment, and it has turned HIV infection from fatal infection to the chronic disease (Coffin *et al.*, 1986; De Clercq, 2006, 2007a, b). HAART uses a combination of various antiviral drugs that inhibit the viral growth at different stages of its life cycle (Gallant *et al.*, 2006; Pozniak *et al.*, 2006). There is continuous need for the development of new drugs as the virus may develop resistance. In the studies of inhibitory effects of chalcones against plant viruses and human rhinoviruses, the antiviral property of chalcones was exploited. The antiviral efficacy is known to depend on the substitution patterns (Nowakowska, 2007). Xanthohumol (Fig. 1) has been reported as an inhibitor of HIV-1 with EC_{50} value of 0.50 μg/ml (Wang *et al.*, 2004). Similarly, chalcone extracted from the genus *Desmos* (Fig. 1) exhibited potent anti-HIV activity with EC_{50} = 0.022 μg/ml (Wu *et al.*, 2003).

Chalcones (1,3-diaryl-2-propen-1-ones) constitute an important class of natural or synthetic compounds belonging to the flavonoids family and are known to exhibit a broad spectrum of various biological activities. The presence of α,β-unsaturated carbonyl moiety as well as of substituted aromatic rings renders the chalcones biologically active. Some substituted chalcones and their heterocyclic derivatives are reported to inhibit the growth of microbes (Sato *et al.*, 1997, Bukhari *et al.*, 2012) and malarial parasites (Henry, 1973). Many chalcones have been claimed to be toxic to various animals (Jeney *et al.*, 1955) and have also shown inhibitory effects on several enzymes (Hase *et al.*, 1973) and herbaceous plants

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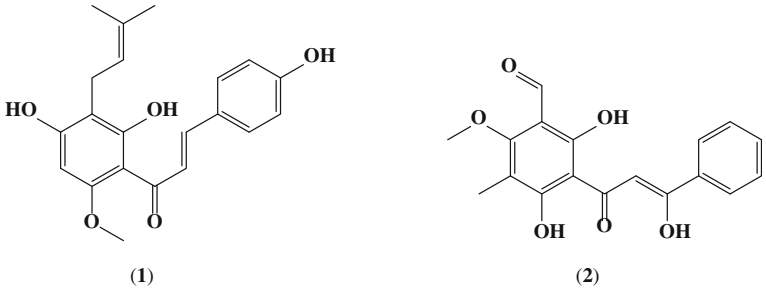


Fig. 1 Structures of anti-HIV chalcones: xanthohumol (**1**), and the one extracted from the genus *Desmos* (**2**)

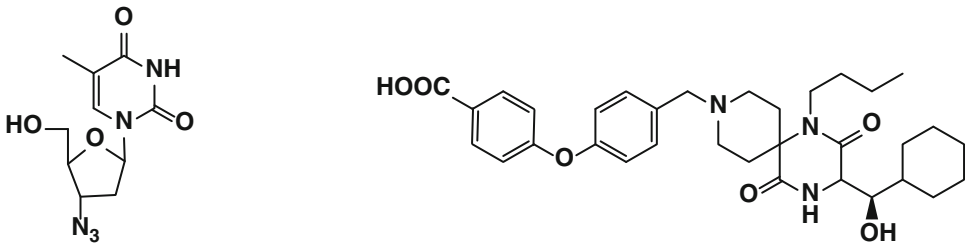


Fig. 2 Structures of potent antiviral drugs, AZT, and aplaviroc

(Cross, 1975). Major biological activities which have been reported to be associated with chalcones include anti-inflammatory (Ballesteros *et al.*, 1995), antifungal (Go *et al.*, 2005), antioxidant (Arty *et al.*, 2000), antimalarial (Narender *et al.*, 2005), antileishmanial (Boeck *et al.*, 2006), antituberculosis (Sivakumar *et al.*, 2005), analgesic (Viana *et al.*, 2003), anti-HIV (Tiwari *et al.*, 1989), anti-tumor (Tiwari *et al.*, 1989; Xia *et al.*, 2000), and cytotoxic (Champelovier *et al.*, 2011).

Our previous studies have exhibited that quinoline-based chalcones display antimicrobial (Rizvi *et al.*, 2010), antileishmanial (Rizvi *et al.*, 2010, 2012a), anti HIV-1 cytotoxic (Rizvi *et al.*, 2012b), and anti-angiogenic activities (Rizvi *et al.*, 2012c). The present study was designed to further investigate the in vitro anti-HIV-1 and cytotoxicity in two series (**4a**–**s** and **5a**–**s**) of quinolyl chalcones. Heterocyclic skeleton plays a key role in the antiviral activity as is depicted by the structures of various antiviral drugs, e.g., Zidovudine (**I**) and Aplaviroc (**II**) (Fig. 2).

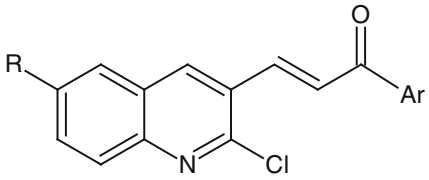
Keeping in view the potential of heterocyclic ring system and chalcones in the antiviral activity, we planned to synthesize the titled chalcones having diversified structural features. The chalcones thus prepared are based on quinoline ring system, and they bear a range of substituted thiophene and furan moieties. The hypothesis of this research was based on the assumption that quinoline- and thiophene-synergized molecules bearing chalcone functionalities are expected to be potent HIV inhibitors. This study resulted in the discovery of 20 new anti-HIV agents (Table 1).

Materials and methods

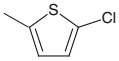
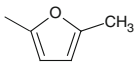
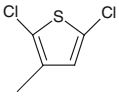
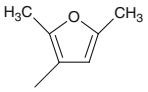
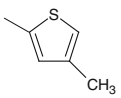
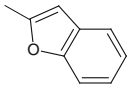
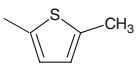
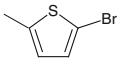
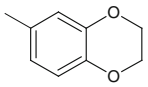
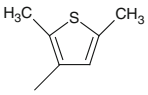
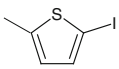
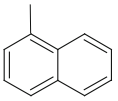
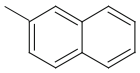
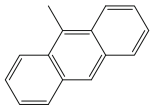
General synthesis of compounds (**4a**–**s** and **5a**–**s**) was carried out from substituted anilines using Vilsmeier–Haack reaction conditions as depicted in Fig. 3 and have been reported previously by our group (Rizvi *et al.*, 2010). The synthesized compounds were screened for their in vitro antiviral effects in human peripheral blood mononuclear (PBM) cells according to our standardized assay (Schinazi *et al.*, 1990). Cells obtained from LifeSouth Community Blood Centers (Atlanta, GA) were isolated by Histopaque (Sigma-Aldrich, St. Louis, MO) and discontinuous gradient centrifugation from healthy seronegative donors. The median effective concentration (EC_{50}) was determined using a reported method (Belen'kii and Schinazi 1994). Assays were conducted using at least two different donor cells in duplicate or triplicate. The results are presented in Table 2.

All the titled compounds were also evaluated for cytotoxicity in human PBM, CEM, and Vero cells, to determine their spectrum of toxicity. CEM cells are a line of lymphoblastic cells originally derived from a child with acute lymphoblastic leukemia whereas; Vero cells are derived from African Green monkey kidney cells. These cells (i.e., PBM, CEM, and Vero cells) were cultured in 96-well plates (5×10^4 cells per well) along with increasing concentrations of the test compound (Stuyver *et al.*, 2002). Cell viability was measured after 5-day incubation period using the Cell Titer 96 Aqueous One Solution cell proliferation assay (Promega, Madison, Wis.) by incubating in

Table 1 Aryl moiety (Ar) of aromatic ketones (**4a–s** and **5a–s**)



(**4a-s**) and (**5a-s**) **4**: R = Me **5**: R = OMe

Chalcones	Ar	Chalcones	Ar	Chalcones	Ar
a		**g**		**m**	
b		**h**		**n**	
c		**i**		**o**	
d		**j**		**p**	
e		**k**		**q**	
f		**l**		**r**	
				s	

an incubator at 37 °C with 5 % CO_2 for human PBM cells. The results are also summarized in Table 2.

Results and discussion

The results (Table 2) indicated that the chalcones derived from unsubstituted thienyl derivatives (**4a** and **5a**) were shown to demonstrate good anti HIV-1 activity (EC_{50} = 5.3 and 9.5 μM, respectively). Thus, introduction of diversified substitution pattern resulted in the establishment of interesting structure–activity relationship. In the series **4a–s**, it is observed that incorporation of electron-donating substituents (by mesomeric effect) to the thiophenyl ring shows activity comparable to **4a**. For example, compounds **4b, 4c, 4d**, and **4e** that have methyl substitutions at various positions show comparable potency to unsubstituted compound **4a** against HIV-1. However, a substitution that is electron withdrawing by inductive effect at *ortho* position reduces the anti-HIV-1 activity drastically, as is the case with **4f** (bearing *ortho* chloro substitution) EC_{50} value >100 μM. Our analogy for loss of potency is that halogens are electron withdrawing by inductive effect and are electron donating by resonance

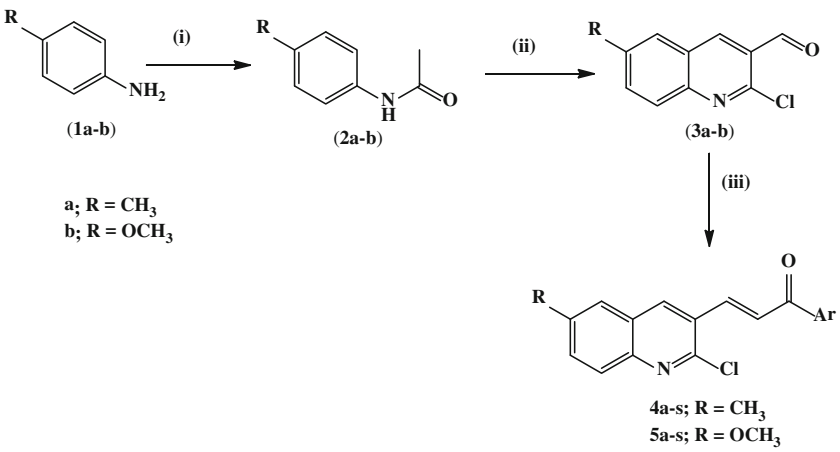


Fig. 3 Schematic lay-out for the synthesis of chalcone compounds (**4a–s** and **5a–s**)

Table 2 Anti-HIV-1 activity in human PBM cells (EC_{50}) and cytotoxic activities in PBM, CEM, and vero cells (IC_{50}) of the series **4a–s** and **5a–s**

Anti-HIV-1 activity in human PBM cells			Cytotoxicity (IC_{50}, μM) in		
Code	EC_{50} (μM)	EC_{90} (μM)	PBM	CEM	Vero
AZT	0.0033 ± 0.022	0.030 ± 0.015	>100	14.3	56.0
4a	5.3	35.0	10.7	3.2	4.8
4b	8.3	22.7	25.3	82.1	66.5
4c	>10	>10	42.9	≥100	64.2
4d	24.4	>100	55.4	4.4	69.1
4e	7.3	18.7	13.0	11.6	2.4
4f	>100	>100	15.8	31.6	95.4
4g	5.7	13.0	25.7	31.6	48.2
4h	9.8	29.1	10.6	11.9	16.9
4i	>10	>10	16.1	31.6	87.9
4j	>10	>10	16.3	31.6	15.5
4k	>10	>10	32.0	61.1	9.8
4l	4.9	11.5	19.9	4.9	11.6
4m	6.6	16.3	10.8	14.0	13.3
4n	50.6	>100	>100	>100	>100
4o	3.2	5.5	4.8	5.5	17.3
4p	>10	>10	43.7	16.5	15.5
4q	>100	>100	>100	16.7	13.0
4r	37.4	100	>100	22.6	35.8
4s	10.5	33.3	83.0	2.9	20.9
5a	9.5	44.5	14.0	1.8	9.1
5b	>10	>10	47.8	10.6	82.9
5c	>10	>10	15.5	15.0	31.6
5d	>10	>10	13.0	14.7	45.9
5e	1.4	4.5	3.9	1.6	8.9
5f	7.9	>10	14.6	2.3	14.2
5g	>10	>10	20.8	31.6	11.6
5h	1.1	6.2	2.7	6.5	4.6
5i	8.1	>10	2.5	8.3	9.5
5j	>10	>10	17.3	31.6	13.2
5k	>10	>10	23.7	71.6	17.6
5l	3.8	20.0	1.7	4.7	16.4
5m	5.4	26.4	4.5	15.7	22.9
5n	>100	>100	>100	>100	>100
5o	2.7	12.0	3.4	10.8	13.7
5p	>10	>10	14.2	31.6	12.1
5q	5.7	38.7	23.2	31.6	14.9
5r	3.8	20.8	>100	>100	52.0
5s	8.0	53.8	10.2	9.8	15.6

effect. Therefore, halogens at the *ortho* position dominate the inductive effect, and as a result, the net effect of the substituents is electron withdrawing, reducing the potency against HIV-1. However, halogen substitution at *meta* and *para* positions exhibited activity comparable with **4a,** e.g., **4g** and **4h**. Similarly, the unsubstituted pyrrole ring demonstrated antiviral activity comparable with **4a** with EC_{50} value of 4.9 μM. Interestingly, the similar behavior is observed for compound **4o** (containing unsubstituted benzofuran ring) which displayed EC_{50} value of 3.2 μM (Fig. 4).

For quinolinyl chalcones **5a–s** bearing methoxy substitution at the quinoline ring system, very interesting and encouraging results were obtained. Compounds **5a, 5l**, and **5o** having no substituent on ring B, i.e., thiophene, pyrrole, and benzofuran ring displayed good biological activity with EC_{50} values of 9.5, 3.8, and 2.7 μM, respectively.

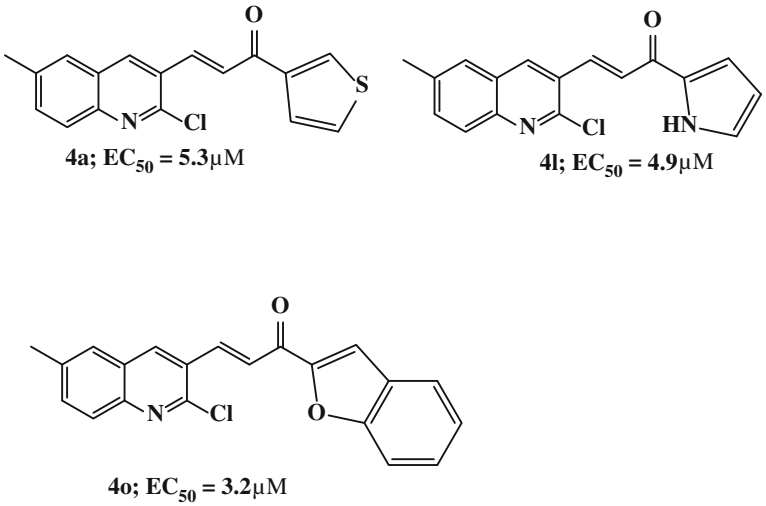


Fig. 4 Anti-HIV-1 activity of quinolinyl chalcones having unsubstituted ring B (thiophene, pyrrole, and benzofuran, respectively)

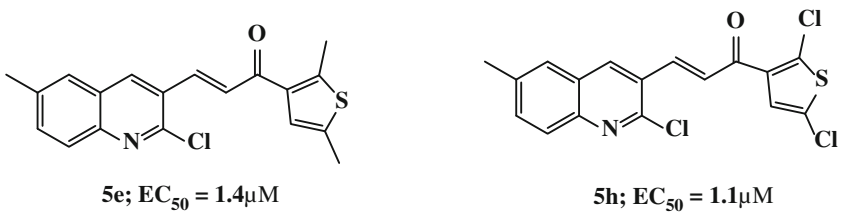


Fig. 5 Structure of the most potent anti-HIV-1 agents **5e** and **5h**, among the titled chalcones

Compounds **5e** and **5h** having 2,5-dimethyl and 2,5-dichlorothiophenyl moieties demonstrated the most potent biological activity with EC_{50} values of 1.4 and 1.1 μM, respectively (Fig. 5).

Among the compounds under investigation, the unsubstituted thiophene derivatives (**4a** and **5a**) showed cytotoxicity in all three types of cells (i.e., PBM, CEM, and Vero cells). Methyl- and halo-substituted derivatives at position 3 (**4b**, **4f**, **4i**, **5b**, **5f,** and **5i**) and position 5 (**4d**, **4g**, **4j–k**, **5d**, **5g**, and **5j–k**), and the chalcone **4d** proved to be nontoxic in human PBM cells, while **4b**, **4k,** and **5k** showed no toxicity in CEM cells and **4b**, **4d**, **4f**, **4i**, and **5b** exhibited no cytotoxicity in Vero cells. The disubstituted thiophene derivatives (**4e**, **4h**, **5e,** and **5h**) were found to be toxic in all three types of cells. Similarly, pyrrole-, 5-methyl furan-, benzofuran-, and benzodioxane derivatives (**4l/5l, 4m/5m**, **4o/5o**, and **4p/5p**, respectively) were cytotoxic to all three types of cells used. In case of naphthalene derivatives, compound (**5q**) proved to be cytotoxic, while **5r** was nontoxic against all three types of cells. Finally, in case of anthracene derivatives of chalcones, compound (**4s**) was proven to be nontoxic in human PBM cells.

In conclusion, two series of quinolyl chalcones (**4a–s** and **5a–s**) were synthesized and tested against HIV-1_{LAI} in primary human PBM cells for antiviral and cytotoxic activities. Seventeen compounds (**4a, 4e**, **4g–h**, **4l–m**, **4o, 5a**, **5e–f**, **5h–i, 5l–m**, **5o, 5q**, and **5s**) were found to be potent against HIV-1 at EC_{50} less than 10 μM and significant cytotoxicity at active concentrations, especially at or less than the antiviral concentrations. Compounds **4o**, **5e**, **5h**, and **5o** demonstrated the most potent anti-HIV-1 activity (EC_{50} = 1.1, 1.4, 2.7, and 3.2 μM, respectively), however, with high toxicity less than 5 μM in human PBM cells. This compounds may serve as a lead for further anti-HIV drug development.

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