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Original article

Synthesis, antimicrobial and antiviral evaluation of substituted imidazole derivatives

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ABSTRACT

In the present study, we have synthesized 2-(substituted phenyl)-1*H*-imidazole (**1**–**12**) and (substituted phenyl)-[2-(substituted phenyl)-imidazol-1-yl]-methanone (**13**–**26**) analogues and screened them for their antimicrobial activity against Gram positive, Gram negative and fungal species. The results of antibacterial study indicated that compounds **15**, **17** and **24** showed appreciable antibacterial activity and compound **26** emerged as the most potential antifungal agent. The results of SAR studies indicated that the presence of electron withdrawing groups is necessary for the antimicrobial activity of the synthesized compounds. The results of the present study indicated that compounds **15**, **17** and **24** might be of interest for the identification of new antimicrobial molecules as their antibacterial activity is equivalent to the standard drug norfloxacin. Further, the antiviral screening of (substituted phenyl)-[2-(substituted phenyl)-imidazol-1-yl]-methanones (**13**–**26**) against a panel of viral strains indicated that compounds **16** and **19** can be selected as lead compounds for the development of novel antiviral agents.

1. Introduction

The incidence of invasive microbial infections caused by opportunistic pathogens, often characterized by high mortality rates, has been increasing over the past two decades. Patients who become severely immunocompromised because of underlying diseases such as leukemia or recently acquired immunodeficiency syndrome or patients who undergo cancer chemotherapy or organ transplantation are particularly susceptible to opportunistic microbial infection [1]. Almost all of the major classes of antibiotics have encountered resistance in clinical applications [2]. The emergence of bacterial resistance to β-lactam antibiotics, macrolides, quinolones and vancomycin is becoming a major worldwide health problem [3].

A matter of concern in the treatment of microbial infections is the limited number of efficacious antimicrobial drugs. Many of the currently available drugs are toxic, enable recurrence because they are bacteriostatic/fungistatic and not bactericidal/fungicidal or lead to the development of resistance due in part to the prolonged periods of administration [4]. There is a real perceived need for the discovery of new compounds that are endowed with antibacterial and antifungal activities, possibly acting through mechanism of actions, which are distinct from those of well known classes of antimicrobial agents to which many clinically relevant pathogens are now resistant [5].

The herpes virus family contains eight known human viruses, amongst them are herpes simplex virus-1 (HSV-1) and herpes simplex virus-2 (HSV-2) that cause mucocutaneous infections resulting in cold sores (HSV-1) and genital lesions (HSV-2), respectively. Much research has been focused on HSV-1 and HSV-2 as these viruses have a high incidence rate and a high prevalence [6]. Vesicular stomatitis virus (VSV), a member of the *Rhabdoviridae* family, is an enveloped single-stranded RNA virus that causes an economically important disease in cattle, horses and swine [7].

The *alphaviruses* are a genus of approximately 27 arthropod-transmitted plus-strand RNA viruses found in the *Togaviridae* family which is responsible for a wide range of diseases and many of them are important human and animal pathogens. Several examples of *alphaviruses* are Sindbis, Semliki Forest, and Venezuelan equine encephalitis viruses. Infection can result in fever, rash, arthralgia or arthritis, lassitude, headache, and myalgia. The prototypic alphavirus is Sindbis virus (SINV), which is transmitted to humans through mosquito bites [8]. Respiratory syncytial virus (RSV), a *paramyxovirus*, is an important cause of respiratory tract infection in infants, young children and adults [9].

Previous antiviral research on herpes simplex viruses has primarily focused on the development of nucleoside analogues,

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such as acyclovir (Zovirax), valacyclovir, famciclovir, and penciclovir. Recently, immunomodulators (imiquimod and resiquimod), nonnucleoside viral polymerase inhibitors (4-hydroxyquinoline-3-carboxamides), and viral helicase inhibitors (thiazolylphenyl and thiazolylamide) have received considerable attention. Though numerous strategies and considerable efforts have been spent in search of the next generation antiviral therapy, it has proved difficult to outperform acyclovir [6,10].

The incorporation of the imidazole nucleus is an important synthetic strategy in drug discovery. The high therapeutic properties of the imidazole related drugs have encouraged the medicinal chemists to synthesize a large number of novel chemotherapeutic agents. Imidazole drugs have broadened scope in remedying various dispositions in clinical medicines. Medicinal properties include anticancer [11], β-lactamase inhibitors [12], 20-HETE synthase inhibitors [13], carboxypeptidase inhibitors [14], hemeoxygenase inhibitors [15], antiaging agents [16], anticoagulants [17], anti-inflammatory [18], antibacterial [19], antifungal [20], antiviral [21], antitubercular [22], antidiabetic [23] and antimalarial [24], all are unique characteristics known for imidazole derivatives.

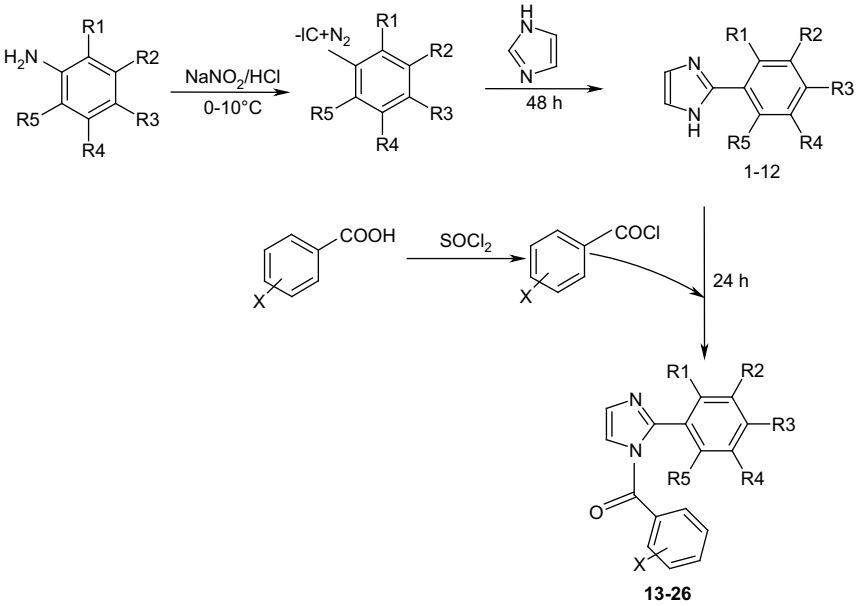
In continuation with our research program concerning the synthesis and antimicrobial evaluation of medicinally important compounds [25–33], in the present study we have synthesized 2-(substituted phenyl)-1*H*-imidazoles (**1**–**12**). Further recent literature revealed that N_1-substitution of imidazoles improves the antibacterial activity [34]. This created interest among us to synthesize (substituted phenyl)-[2-(substituted phenyl)-imidazol-1-yl]-methanones (**13**–**26**) and screen them for their antimicrobial and antiviral activity.

2. Chemistry

Synthesis of compounds **1**–**26** followed the general pathway that is depicted in Scheme 1. Compounds **1**–**12** are the key intermediates for the synthesis of compounds **13**–**26**. The key intermediates, 2-(substituted phenyl)-1*H*-imidazoles (**1**–**12**), were prepared by the condensation of imidazoles with corresponding substituted aryldiazonium chlorides which in turn were prepared by the diazotization of substituted anilines. However, based on our experience, the application of cupric chloride to the condensation of aryldiazonium chloride with benzimidazole as suggested by Dahiya and Pathak [35] resulted in resinous products. Therefore, coupling was carried out using sodium acetate along with stirring in cold conditions for the initial 3 h followed by 48 h stirring at room temperature which resulted in a solid product. For the synthesis of (substituted phenyl)-[2-(substituted phenyl)-imidazol-1-yl]-methanones (**13**–**26**), the key intermediates (**1**–**12**) were reacted with substituted benzoyl chloride which was prepared by the reaction of substituted benzoic acid with thionyl chloride. The physicochemical characteristics of the synthesized compounds are presented in Table 1.

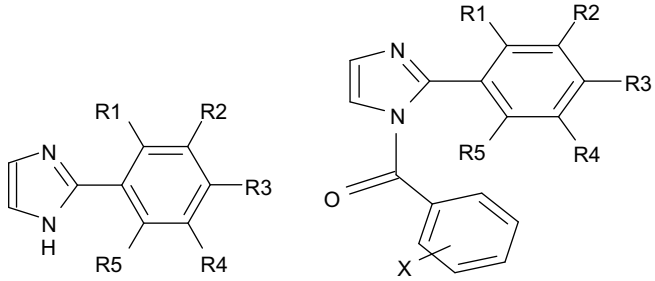
The structures of compounds **1**–**26** were assigned by IR and ^{1}H NMR spectroscopic data, which are consistent with the proposed molecular structures. The appearance of medium out-of-plane deformation bands (C–C bending) at 725–680 cm^{-1} indicated the presence of 1,3-disubstituted benzene ring in compounds **3** and **18**. Similarly, the appearance of the C–C out-of-plane band at 790–810 cm^{-1} indicated the presence of 1,4-disubstituted benzene ring in compounds **2**, **6**, **13**, **17**, **18** and **25**. The presence of 2-substituted benzene in structures of compounds **17**, **23**, and **25** was confirmed by strong out-of-plane deformation bands (C–H bending) around 740 cm^{-1} which were visible from their IR spectra. Moreover, the presence of NO_2 group in compounds **4**, **6**, **13**, **17** and **18** was indicated by the appearance of asymmetric and symmetric NO_2 stretching bands at 1550–1510 cm^{-1} and 1365–1335 cm^{-1}, respectively. The appearance of medium bands at 3696.2 cm^{-1} in the IR spectra of compound **17** indicated the presence of a free OH in the carboxylic acid group of 2-substituted benzene. Further, the appearance of strong C=O stretching bands at 1695–1670 cm^{-1} in the IR spectra of (substituted phenyl)-[2-(substituted phenyl)-imidazol-1-yl]-methanones (**13**–**26**) demonstrated the presence of tertiary amide linkage between substituted benzoic acid and imidazole nucleus. Compounds **23** and **25** showed C–Br stretching at 522.8 cm^{-1} and 533.8 cm^{-1}, which confirms the presence of 2-bromo substituted benzene ring. The presence of aliphatic CH stretching at 2923.2 cm^{-1} and CH stretching of aralkyl ether at 3054.5 cm^{-1} in compound **25** confirms the existence of OCH_3 group stretching.

Compounds **13** and **17** showed multiplet signal at δ 7.69–7.77 ppm corresponding to the protons of substituted benzene at C_2 of imidazole. In contrast, compounds **18** and **23** showed the multiplet signals at δ 6.98–7.42 ppm for the protons of substituted



Scheme 1. Synthetic scheme for the synthesis of 2-(substituted phenyl)-1*H*-imidazoles and (substituted phenyl)-[2-(substituted phenyl)-imidazol-1-yl]-methanones.

Table 1
Physicochemical characteristics of synthesized 2-(substituted phenyl)-1*H*-imidazoles and (substituted phenyl)-[2-(substituted phenyl)-imidazol-1-yl]-methanones



1-12 **13-26**

Compound	R_1	R_2	R_3	R_4	R_5	X	Mol. formula	Mol. wt	Mp (°C)	R_f	%Yield
1	Cl	H	H	H	H	–	$C_9H_7N_2Cl$	178.66	100	0.80[a]	28.67
2	H	H	Cl	H	H	–	$C_9H_7N_2Cl$	178.66	>250	0.86[a]	59.13
3	H	Cl	H	H	H	–	$C_9H_7N_3O_2$	189.23	196–199	0.41[a]	12.45
4	H	NO_2	H	H	H	–	$C_9H_7N_3O_2$	189.23	201–203	0.81[a]	53.13
5	NO_2	H	H	H	H	–	$C_9H_7N_2Cl$	178.66	99–101	0.80[a]	44.31
6	H	H	NO_2	H	H	–	$C_9H_8N_2$	144.17	51–53	0.60[a]	34.65
7	H	H	H	H	H	–	$C_9H_7N_3O_2$	189.23	>250	0.20[a]	47.07
8	COOH	H	H	H	H	–	$C_{10}H_8N_2O_2$	188.24	>250	0.12[a]	41.79
9	H	H	OCH_3	H	H	–	$C_{10}H_{10}N_2O_2$	190.25	109–111	0.29[a]	57.89
10	CH_3	CH_3	H	H	H	–	$C_{11}H_{12}N_2$	172.23	219–221	0.71[b]	43.70
11	CH_3	H	H	CH_3	CH_3	–	$C_{11}H_{12}N_2$	172.23	209–211	0.53[b]	74.32
12	H	H	Br	H	H	–	$C_9H_7BrN_2$	123.07	>250	0.2[b]	53.61
13	H	NO_2	H	H	H	4-NO_2	$C_{16}H_{10}N_4O_5$	338.28	139–141	0.13[a]	35.73
14	NO_2	H	H	H	H	4-NO_2	$C_{16}H_{10}N_4O_5$	338.28	149–151	0.14[a]	41.09
15	Cl	H	H	H	H	4-NO_2	$C_{16}H_{10}N_3O_3Cl$	327.73	221–223	0.11[a]	56.07
16	H	H	Cl	H	H	4-NO_2	$C_{16}H_{10}N_3O_3Cl$	327.73	219–221	0.33[a]	65.72
17	COOH	H	H	H	H	4-NO_2	$C_{17}H_{11}N_3O_5$	337.29	194–196	0.34[a]	19.56
18	H	Cl	H	H	H	4-NO_2	$C_{16}H_{10}ClN_3O_3$	327.73	214–216	0.11[a]	76.54
19	H	H	NO_2	H	H	4-NO_2	$C_{16}H_{10}N_4O_5$	338.28	209–211	0.66[a]	59.80
20	H	H	OCH_3	H	H	4-NO_2	$C_{17}H_{13}N_3O_4$	323.31	229–231	0.10[a]	78.65
21	NO_2	H	H	H	H	2-Br	$C_{16}H_{10}BrN_3O_3$	372.18	119–121	0.85[a]	60.98
22	H	NO_2	H	H	H	2-Br	$C_{16}H_{10}BrN_3O_3$	372.18	114–116	0.73[a]	58.65
23	Cl	H	H	H	H	2-Br	$C_{16}H_{10}BrClN_2O$	361.63	149–151	0.85[a]	64.23
24	H	H	Cl	H	H	2-Br	$C_{16}H_{10}BrClN_2O$	361.63	139–141	0.82[a]	38.65
25	H	H	OCH_3	H	H	2-Br	$C_{17}H_{17}BrN_2O_2$	357.21	139–141	0.82[a]	75.86
26	H	H	NO_2	H	H	2-Br	$C_{16}H_{10}BrN_3O_3$	372.18	109–111	0.78[a]	49.78

[a] Toluene:chloroform (7:3).
[b] Benzene.

benzene at C_2 of imidazole. This may be due to the interchange of electron withdrawing groups in compounds **13** and **17** to electron donating groups in compounds **18** and **23**. Compounds **13**, **17** and **18** showed signals at δ 8.07–8.39 ppm due to the presence of the *p*-NO_2 acyl substituent at N_1 of imidazole but in compounds **23** and **25** a downfield shift at δ 7.50–7.59 ppm due to the substitution of the electron withdrawing NO_2 with bromine was observed. In compounds **13** and **18** signal corresponding to C_4 of imidazole was observed at δ 7.33 ppm but in compounds **17** and **23** the signal of C_4 of imidazole was seen at δ 7.15 ppm. This effect may probably be due to the presence of the 3-substituted benzene in compounds **13** and **18** and 2-substituted benzene in compounds **17** and **23**. The appearance of signal at δ 3.90 ppm confirmed the presence of OCH_3 group in compound **25**.

3. Results and discussion

3.1. Antimicrobial activity

The synthesized compounds were screened for their in vitro antimicrobial activities against two Gram-positive bacteria – *Staphylococcus aureus*, *Bacillus subtilis*; Gram-negative bacterium – *Escherichia coli* and fungal strains – *Aspergillus niger* and *Candida albicans* by the tube dilution method [36] using norfloxacin and fluconazole as control drugs for antibacterial and antifungal activities, respectively. The results of the antimicrobial studies are presented in Table 2. In general the compounds showed improved antibacterial activity when compared to their antifungal activity. The deduced patterns of antimicrobial activity of substituted imidazoles are in the following order: antibacterial > antifungal.

All of the synthesized substituted imidazole derivatives showed appreciable in vitro antimicrobial activity against the tested microorganisms. Compounds **15**, **17**, **20**, **21**, **24** and **26** were found to be highly active against *S. aureus* showing a bacterial inhibition at 2×10^{-3} μM/ml. The synthesized derivatives **15**, **17**, **20**, **21** and **24** showed high antibacterial potential at 2×10^{-3} μM/ml against *B. subtilis*. The antibacterial potential against *E. coli* was exhibited by compounds **15**, **17**, **22** and **24** at concentrations ranging from 2×10^{-3} μM/ml to 18×10^{-3} μM/ml. Compounds **14**, **16**, **21** and **26** were found to be effective against *A. niger* having MIC of 4×10^{-3} μM/ml. Compounds **22**, **25** and **26** were found to possess high antifungal potential against *C. albicans* when compared to other derivatives.

The most active antibacterial agents, **15**, **17** and **24**, with MIC value of 2×10^{-3} μM/ml have activities equivalent to that of the standard drug norfloxacin (MIC = 2×10^{-3} μM/ml). Further modification of the above structures may provide compounds with better antibacterial potential than the reference compound, norfloxacin.

Compound **21** emerged as the most effective antibacterial agent against all of the tested microorganisms, except for *E. coli* and *C. albicans*, based on its MIC values but it failed to prove effective against the tested strains on the basis of its MBC and MFC values (Table 3).

Table 2
Antimicrobial activity of synthesized 2-(substituted phenyl)-1*H*-imidazoles and (substituted phenyl)-[2-(substituted phenyl)-imidazol-1-yl]-methanones

Compound	Minimum inhibitory concentration (1×10^{-3} μM/ml)				
	S. aureus	*B. subtilis*	*E. coli*	*A. niger*	*C. albicans*
1	35	702	702	35	702
2	4	8	8	702	35
3	4	17	702	35	35
4	4	4	4	33	33
5	4	4	4	33	33
6	4	16	4	33	33
7	5	5	5	86	43
8	4	4	4	33	66
9	4	4	32	65	65
10	4	4	4	17	72
11	4	4	72	9	36
12	3	3	3	14	28
13	18	36	9	9	18
14	36	36	36	4	36
15	2	2	2	9	38
16	38	19	38	4	19
17	2	2	2	9	18
18	38	38	38	19	38
19	73	36	73	18	18
20	2	2	18	9	18
21	2	2	33	4	33
22	4	33	2	16	16
23	69	69	69	8	17
24	2	2	2	8	17
25	16	16	33	16	16
26	2	4	4	4	16
Std	2[a]	2[a]	2[a]	1[b]	1[b]

[a] Norfloxacin.
[b] Fluconazole.

Table 3
MBC/MFC values of synthesized 2-(substituted phenyl)-1*H*-imidazoles and (substituted phenyl)-[2-(substituted phenyl)-imidazol-1-yl]-methanones

Compound	Minimum bactericidal/fungicidal concentration (1×10^{-3} μM/ml)				
	S. aureus	*B. subtilis*	*E. coli*	*A. niger*	*C. albicans*
1	561	561	561	280	561
2	35	702	702	561	280
3	35	140	561	280	280
4	33	33	33	264	264
5	33	33	33	264	264
6	33	132	33	264	264
7	43	43	43	694	347
8	531	33	265	265	526
9	32	32	263	526	526
10	36	36	36	144	289
11	36	36	578	144	289
12	28	28	28	112	224
13	147	295	36	73	295
14	295	295	295	73	295
15	19	19	19	76	152
16	152	305	305	76	152
17	18	18	18	148	256
18	305	305	305	152	304
19	295	295	295	147	147
20	18	18	147	147	147
21	268	268	268	67	268
22	33	268	16	67	268
23	268	268	268	138	138
24	17	17	17	69	138
25	134	628	628	134	134
26	16	33	33	67	134
Std	19[a]	19[a]	19[a]	40[b]	40[b]

[a] Norfloxacin.
[b] Fluconazole.

In general, the MFC and MBC values of the synthesized imidazole derivatives were 3-fold higher than the MIC values which indicated that the synthesized compounds were bacteriostatic and fungistatic in action (a drug is considered to be bacteriostatic/fungistatic when its MFC and MBC values are 3-fold higher than its MIC value) [37].

3.2. Structure activity relationship (SAR) studies

From the results of the antimicrobial activity of the synthesized substituted imidazole derivatives, the following structure activity relationships can be derived:

(i) N_1-Substituted imidazole derivatives exhibited high antimicrobial activity in comparison to N_1-non-substituted imidazole derivatives. This was similar to the literature reports [1,34] where little or no activity was found for the non-N_1-substituted derivatives of benzimidazoles and imidazoles.

(ii) Compound **17**, having COOH substitution i.e. 2-[1-(4-nitrobenzoyl)-1*H*-imidazol-2-yl]-benzoic acid, showed profound antibacterial activity without any significant activity against tested fungal strains. The significant antibacterial activity (2×10^{-3} μM/ml) may be attributed to the presence of the COOH group which is also present in the standard drug norfloxacin. The lower antifungal activity is supported by the work of Goker et al. [1] who reported that the presence of trifluoromethyl, carboxylic, ester and amide groups does not improve antifungal activity.

(iii) In general, it was observed that most of the compounds with substituted phenyl rings showed better antimicrobial activity than the ones with a non-substituted phenyl ring (compound **7**). This was similar to the results observed by Tekiner-Gulbas et al. [38] who stated that the *p*-substituted phenyl causes an increase in activity than the unsubstituted phenyl ring in the case of oxazoles.

(iv) The high antimicrobial activity of compounds **13**–**26** in comparison to compounds **1**–**12** may be attributed to the presence of carbonyl group in the former. This is supported by the fact that removal of carbonyl group in bis-imidazole derivatives resulted in loss of their antifungal activity [39].

(v) Compounds **15**, **17** and **24** were found to be the most effective antibacterial agents, whereas compound **26** emerged as the most effective antifungal agent. This indicated that there are different structural requirements for binding of drug to bacterial or fungal targets, respectively [40].

(vi) In general, the most active compounds contain an electron withdrawing substituent on phenyl ring at second position of imidazole. The role of electron withdrawing group in improving antimicrobial activities is supported by the studies of Sharma et al. [41].

(vii) In contrast to point (vi) mentioned above, the presence of electron donating methoxy group also showed significant activity against *S. aureus*, *B. subtilis* and *C. albicans*. The role of methoxy group in improving antifungal activity of imidazoles is supported by the studies of Emami et al. [20].

(viii) As far as antifungal activity against *A. niger* is concerned, the N_1-substituted phenyl ring have both 4-NO_2 and 2-Br substituents as electron withdrawing groups (**14**, **16**, **21** and **26**) whereas in case of *C. albicans* the N_1-substituted phenyl ring has only a bromine substituent as an electron withdrawing moiety. The results indicated that the presence of the electron withdrawing NO_2 substituent on the N_1-substituted phenyl ring may not be favourable for the binding of compounds **22**, **25** and **26** with the fungal target in case of *C. albicans*. The above SAR results are summarized in Fig. 1.

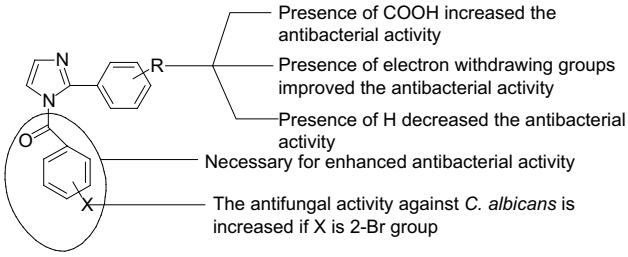


Fig. 1. SAR for antimicrobial activity of (substituted phenyl)-[2-(substituted phenyl)-imidazol-1-yl]-methanones.

3.3. Antiviral activity

The results of antiviral screening of (substituted phenyl)-[2-(substituted phenyl)-imidazol-1-yl]-methanones (**13**–**26**) against herpes simplex virus-1 (KOS) (HSV-1 KOS), herpes simplex virus-2 (G) (HSV-2G), vaccinia virus (VV), vesicular stomatitis virus (VSV), herpes simplex virus-1 TK$^-$ KOS ACV^r (HSV-1 TK$^-$ KOS ACV^r) in HEL cell cultures, vesicular stomatitis virus (VSV), Coxsackie virus B4 (CV-B4), respiratory syncytial virus (RSV) in HeLa cell cultures and parainfluenza-3 virus (PI-3V), reovirus-1 (RV-1), Sindbis virus (SV), Coxsackie virus B4 (CV-B4), Punta Toro virus (PTV) in Vero cell cultures were determined using a CPE reduction assay [42].

Compound **19** emerged as the best antiviral agent against HSV-1 KOS ($EC_{50} = 59$ μg/ml, Table 4), HSV-2G ($EC_{50} = 50$ μg/ml, Table 4) and HSV-1 TK$^-$ KOS ACV^r ($EC_{50} = 45$ μg/ml, Table 4) among the tested (substituted phenyl)-[2-(substituted phenyl)-imidazol-1-yl]-methanones (**13**–**26**), but it was much less active than the reference compounds brivudin, cidofovir and ganciclovir. Against VSV none of the tested compounds were active in HEL cell culture. Further, compounds **16** and **19** (having a *p*-substituted electron withdrawing group at both 1 and 2 positions of imidazole) emerged as the most promising antiviral agents against vaccinia virus tested in HEL cell culture with an EC_{50} value of 2 and 4 μg/ml, respectively (Table 4) and their anti-VV activity was equivalent to that of the standard drugs brivudin ($EC_{50} = 5$ μg/ml) and cidofovir ($EC_{50} = 7$ μg/ml). Compounds **16** and **19** inhibited the viral replication of vaccinia virus at a concentration of 2 μg/ml and 4 μg/ml, respectively, which was almost 50 times and 25 times less than that of their cytotoxic concentration (>100 μg/ml) which made them to be considered as compounds having a marked therapeutic index and be selected as lead compounds for the synthesis of newer antiviral agents. All other compounds tested in HEL cell culture were active at a concentration which caused microscopically detectable alteration of normal cell morphology.

In HeLa cell cultures (Table 5) none of the compounds proved active against any of the tested strains of viruses (VSV, CV-B4, RSV) except compound **19** which showed appreciable antiviral activity against the all aforesaid viruses in HeLa cell culture at an $EC_{50} > 20$ μg/ml, which was ≥5 times lower than its cytotoxic concentration (100 μg/ml).

In Vero cell culture no activity was noted with any of the compounds against parainfluenza-3 virus, reovirus-1, Sindbis virus,

Table 4
Cytotoxicity and antiviral activity of (substituted phenyl)-[2-(substituted phenyl)-imidazol-1-yl]-methanones in HEL cell cultures

Compound	Minimum cytotoxic concentration[a] (μg/ml)	EC_{50}[b] (μg/ml)				
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Vaccinia virus	Vesicular stomatitis virus	Herpes simplex virus-1 TK$^-$ KOS ACV^r
13	>100	>100	>100	>100	>100	>100
14	>100	>100	>100	>100	>100	>100
15	>100	>100	>100	>100	>100	>100
16	>100	>100	>100	2	>100	>100
17	>100	>100	>100	>100	>100	>100
18	>100	>100	>100	45	>100	>100
19	>100	59	50	4	>100	45
20	>100	>100	>100	>100	>100	>100
21	>100	>100	>100	>100	>100	>100
22	>100	>100	>100	>100	>100	>100
23	>100	>100	>100	>100	>100	>100
24	>100	>100	>100	>100	>100	>100
25	>100	>100	>100	>100	>100	>100
26	>100	>100	>100	>100	>100	>100
Brivudin	>250	0.04	29	5	>250	96
Ribavirin	>250	>250	>250	>250	146	>250
Cidofovir	>250	3	2	7	>250	2
Ganciclovir	>100	0.06	0.06	>100	>100	1

[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50%.

Table 5
Cytotoxicity and antiviral activity of (substituted phenyl)-[2-(substituted phenyl)-imidazol-1-yl]-methanones in HeLa cell cultures

Compound	Minimum cytotoxic concentration[a] (μg/ml)	EC_{50}[b] (μg/ml)		
		Vesicular stomatitis virus	Coxsackie virus B4	Respiratory syncytial virus
13	>100	>100	>100	>100
14	>100	>100	>100	>100
15	>100	>100	>100	>100
16	>100	>100	45	>100
17	>100	>100	>100	>100
18	>100	>100	>100	>100
19	100	>20	>20	>20
20	>100	>100	>100	>100
21	>100	>100	>100	>100
22	>100	>100	>100	>100
23	>100	>100	>100	>100
24	>100	>100	>100	>100
25	>100	>100	>100	>100
26	>100	>100	>100	>100
DS-5000	>100	>100	9	0.8
(*S*)-DHPA	>250	>250	>250	>250
Ribavirin	>250	29	146	10

[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50%.

Table 6
Cytotoxicity and antiviral activity of (substituted phenyl)-[2-(substituted phenyl)-imidazol-1-yl]-methanones in VERO cell cultures

Compound	Minimum cytotoxic concentration[a] (μg/ml)	EC_{50}[b] (μg/ml)				
		Parainfluenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie virus B4	Punta Toro virus
13	>100	>100	>100	>100	>100	100
14	>100	>100	>100	>100	>100	>100
15	>100	>100	>100	>100	>100	>100
16	>100	>100	>100	>100	>100	>100
17	>100	>100	>100	>100	>100	>100
18	>100	>100	>100	>100	>100	>100
19	>100	>100	>100	>100	>100	>100
20	>100	>100	>100	>100	>100	>100
21	>100	>100	>100	>100	>100	>100
22	>100	>100	>100	>100	>100	>100
23	>100	>100	>100	>100	>100	>100
24	>100	>100	>100	>100	>100	>100
25	>100	>100	>100	>100	>100	>100
26	>100	>100	>100	>100	>100	>100
DS-5000	>100	>100	>100	59	>100	100
(*S*)-DHPA	>250	>250	>250	>250	>250	>250
Ribavirin	>250	45	>250	>250	>250	146

[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50%.

Coxsackie virus B4, Punta Toro virus at an EC_{50} value of 100 μg/ml (Table 6). Of some importance, no cytotoxicity was noted with any of the compounds in Vero cells.

4. Conclusion

During an effort to discover new 2-(substituted phenyl)-1*H*-imidazoles and (substituted phenyl)-[2-(substituted phenyl)-imidazol-1-yl]-methanone analogues with antimicrobial activity we found that compounds **15**, **17** and **24** showed appreciable antibacterial activity. Compounds **14**, **16**, **21** and **26** were found to be significantly active against *A. niger*. Compounds **22**, **25** and **26** were found to have antifungal activity against *C. albicans*. The results of SAR studies indicated that the presence of electron withdrawing groups is necessary for the antimicrobial activity of the synthesized compounds. Further, the results of the present study indicated that compounds **15**, **17**, **24** and **26** might be of interest for the development of new antimicrobial molecules. Especially, it could be noticed that most of the compounds were more active as antibacterials than as antifungals, which could be of some guidance to design new lead antibacterial compounds. In fact, the most active antibacterial compounds (**15**, **17** and **24**) showed antibacterial activity equivalent to that of the standard drug norfloxacin in both MIC and MBC determinations. Further, the antiviral screening of (substituted phenyl)-[2-(substituted phenyl)-imidazol-1-yl]-methanones (**13**–**26**) against a panel of viral strains indicated that compounds **16** and **19** could be selected as lead compounds for the development of novel antiviral agents active against pox viruses.

5. Experimental

Melting points were determined in open capillary tubes on a Sonar melting point apparatus and are uncorrected. Reaction progress was monitored by thin layer chromatography on silica gel sheets (Merck silica gel-G) and the purity of the compounds was ascertained by single spot on TLC sheet. ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker Avance II 400 NMR spectrometer using appropriate deuterated solvents and are expressed in parts per million (δ, ppm) downfield from tetramethylsilane (internal standard). Infrared (IR) spectra were recorded on a Shimadzu FTIR spectrometer.

5.1. General procedure for the synthesis of 2-(substituted phenyl)-1H-imidazoles (**1**–**12**)

Substituted anilines (0.13 mol) in hydrochloric acid/water mixture (1:1) were diazotized using solution of sodium nitrite at 0–10 °C. To the diazotized mixture, imidazole (0.004 mol) was added with vigorous shaking. A solution of sodium acetate (40 g in 100 ml) was added dropwise to the above mixture by maintaining the temperature at 5–10 °C. The above solution was stirred initially for 3 h at cold condition followed by continuation of stirring at room temperature for 48 h. The product obtained was filtered, dried and recrystallized from alcohol.

5.2. General procedure for the synthesis of (substituted phenyl)-[2-(substituted phenyl)-imidazol-1-yl]-methanones (**13**–**26**)

A solution of 2-(substituted phenyl)-1*H*-imidazoles (**1**–**12**) (0.002 mol) in diethyl ether (50 ml) was added to a solution of corresponding substituted benzoyl chloride (0.002 mol) in diethyl ether (50 ml). The above mixture was stirred for 24 h at room temperature. The resultant product was isolated by evaporation of ether and purified by recrystallization with methanol.

Analytical data for compound ***2***: mp (°C) – 100; yield – 44.31%; ^{1}H NMR ($CDCl_3$): δ 7.00–7.03 (d, 2H, CH of C_4 and C_5 of imidazole), 7.20–7.28 (m, 1H, CH of C_4 of ArH), 7.34–7.37 (d, 1H, CH of C_6 of ArH), 7.50 (s, 1H, CH of C_2 of ArH); IR (KBr pellets) cm^{-1}: 3488.02 (NH str, imidazole), 1507.27 (C–C str, Ar), 1660.60 (C–H str, Ar), 738.69 (C–Cl str).

Analytical data for compound ***3***: mp (°C) – 197; yield – 12.45%; ^{1}H NMR ($CDCl_3$): δ 6.55–6.57 (d, 2H, CH of C_4 and C_5 of imidazole), 8.15–8.17 (d, 2H, CH of C_3 and C_5 of ArH), 7.54–7.56 (d, 2H, CH of C_2 and C_6 of ArH); IR (KBr pellets) cm^{-1}: 3481.27 (NH str, imidazole), 1519.80 (C–C str, Ar), 1653.52 (C–H str, Ar), 1342.25 (NO_2 sym, str).

Analytical data for compound ***6***: mp (°C) – >250; yield – 59.13%; ^{1}H NMR ($CDCl_3$): δ 7.21–7.26 (d, 2H, CH of C_4 and C_5 of imidazole), 7.34–7.36 (d, 2H, CH of C_3 and C_5 of ArH), 7.45–7.47 (d, 2H, CH of C_2 and C_6 of ArH); IR (KBr pellets) cm^{-1}: 3489.95 (NH str, imidazole), 1521.73 (C–C str, Ar), 1653.85 (C–H str, Ar), 749.29 (C–Cl str).

Analytical data for compound ***13***: mp (°C) – 139–141; yield – 35.73%; ^{1}H NMR ($CDCl_3$): δ 7.69–7.77 (m, 3H, CH of C_5 of imidazole and CH of C_5 and C_6 of *m*-$ArNO_2$), 7.33 (d, 1H, CH of C_4 of imidazole), 8.19–8.39 (m, 6H, CH of $ArNO_2$); IR (KBr pellets) cm^{-1}: 1604.4 (three ring stretching bands, imidazole), 1540.8 (NO_2 asym str, aromatic NO_2), 1349.6 (NO_2 sym str, aromatic NO_2), 715.8 (C–C out-of-plane bending, 1,3-disubstituted benzene ring), 800.3 (CH out-of-plane bending, 1,4-disubstituted benzene), 1694.0 (C=O str).

Analytical data for compound ***17***: mp (°C) – 194–196; yield – 19.56%; ^{1}H NMR ($CDCl_3$): δ 7.69–7.71 (m, 4H, CH of ArCOOH), 7.15 (d, 1H, CH of C_4 of imidazole), 8.07–8.38 (m, 4H, CH of $ArNO_2$), 7.85 (d, 1H, CH of C_5 of imidazole); IR (KBr pellets) cm^{-1}: 1604.3 (three ring stretching bands, imidazole), 1540.6 (asym str, aromatic NO_2), 1349.8 (sym str, aromatic NO_2), 930.7 (OH out-of-plane bending, COOH), 1290.0 (OH in plane bending, COOH), 3062.1 (CH str, benzene), 799.6 (CH out-of-plane bending, 1,4-disubstituted benzene), 3696.2 (OH str, COOH), 1693.2 (C=O str).

Analytical data for compound ***18***: mp (°C) – 214–216; yield – 76.54%; ^{1}H NMR ($CDCl_3$): δ 8.21–8.26 (m, 4H, CH of $ArNO_2$), 7.33 (d, 1H, CH of C_4 of imidazole), 6.98–7.31 (m, 4H, CH of ArCl); IR (KBr pellets) cm^{-1}: 1601.7 (three ring stretching bands, imidazole), 714.2 (C–C out-of-plane bending, 1,3-disubstituted benzene ring), 1536.2 (NO_2 asym str, aromatic NO_2), 1348.3 (NO_2 sym str, aromatic NO_2), 797.0 (CH out-of-plane bending, 1,4-disubstituted benzene), 1694.4 (C=O str).

Analytical data for compound ***23***: mp (°C) – 149–151; yield – 64.23%; ^{1}H NMR ($CDCl_3$): δ 7.16–7.42 (m, 4H, CH of ArCl), 7.10 (d, 1H, CH of C_4 of imidazole), 7.50–7.70 (m, 4H, CH of ArBr), 7.87 (d, 1H, CH of C_5 of imidazole); IR (KBr pellets) cm^{-1}: 1586.4 (three ring stretching bands, imidazole), 1683.8 (C=O str, Aryl COOH), 3010.30 (CH str, aromatic), 522.2 (C–Br str, aromatic), 684.0 (C–Cl str), 740.7 (CH out-of-plane bending, 1,2 disubstituted benzene ring), 791.1 (CH out-of-plane bending, 1,4-disubstituted benzene).

Analytical data for compound ***25***: mp (°C) – 144–146; yield – 75.86%; ^{1}H NMR ($CDCl_3$): δ 7.40–7.44 (m, 4H, CH of $ArOCH_3$), 7.15 (d, 1H, CH of C_4 of imidazole), 7.59–7.79 (m, 4H, CH of ArBr), 7.87 (d, 1H, CH of C_5 of imidazole), 3.90 (s, 3H, OCH_3); IR (KBr pellets) cm^{-1}: 1587.6 (three ring stretching bands, imidazole), 2923.2 (CH str, CH_3), 3054.5 (CH str, $ArOCH_3$), 533.8 (C–Br str, aromatic), 685.4 (C–Cl str), 741.4 (CH out-of-plane bending, 1,2 disubstituted benzene ring), 810.9 (CH out-of-plane bending, 1,4-disubstituted benzene ring), 1684.1 (C=O str).

5.3. Evaluation of antimicrobial activity

5.3.1. Determination of MIC

The antimicrobial activity was performed against Gram-positive bacteria: *S. aureus*, *B. subtilis*, Gram-negative bacterium: *E. coli* and fungal strains: *C. albicans* and *A. niger*. The standard and test samples were dissolved in DMSO to give a concentration of 100 μg/ml. The minimum inhibitory concentration (MIC) was determined by tube dilution method. Dilutions of test and standard compounds

were prepared in double strength nutrient broth I.P. (bacteria) or Sabouraud dextrose broth I.P. [43] (fungi). The samples were incubated at 37 °C for 24 h (bacteria), at 25 °C for 7 d (*A. niger*) and at 37 °C for 48 h (*C. albicans*), respectively, and the results were recorded in terms of MIC (the lowest concentration of test substance which inhibited the growth of microorganisms).

5.3.2. Determination of MBC/MFC

The minimum bactericidal concentration (MBC) and fungicidal concentration (MFC) were determined by subculturing on fresh medium 100 μL of culture from each tube that remained clear in the MIC determination. MBC and MFC values represent the lowest concentration of compound that produces a 99.9% end point reduction [44].

5.4. Evaluation of antiviral activity

5.4.1. Antiviral assay

The antiviral activity of the (substituted phenyl)-[2-(substituted phenyl)-imidazol-1-yl]-methanones (**13**–**26**) was determined using a CPE reduction assay [42] against herpes simplex virus-1 (KOS), herpes simplex virus-2 (G), vaccinia virus, vesicular stomatitis virus, herpes simplex virus-1 TK^- KOS ACV^r in HEL cell cultures, vesicular stomatitis virus (VSV), Coxsackie virus B4, respiratory syncytial virus in HeLa cell cultures and parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4, Punta Toro virus in Vero cell cultures and the results were expressed as the 50% effective concentration (EC_{50}). Cells, grown to confluency in 96-well plates, were infected with 100 $CCID_{50}$ of virus, one $CCID_{50}$ being the 50% cell culture infective dose. After an adsorption period of 2 h at 37 °C, virus was removed and serial dilutions of the compounds were added. The cultures were further incubated at 37 °C for 3 days, until complete CPE was observed in the infected and untreated virus control.

5.4.2. Cytotoxic assay

The cytotoxicity of the compounds was evaluated in parallel with their antiviral activity in uninfected cells, and is expressed as minimum cytotoxic concentration to cause a microscopically detectable alteration of normal cell morphology (HEL cells, HeLa cells, and Vero cells).

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