RESEARCH ARTICLE

PASS Predication, Antiviral, *in vitro* Antimicrobial, and ADMET Studies of Rhamnopyranoside Esters

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Abstract
Sugar derived esters (SEs) with potential antimicrobial activity were found to be a better choice to solve the multidrug resistant (MDR) pathogens due to improved antimicrobial efficacy, biodegradability, non-toxic, and non-allergic properties. In this context, a series of benzyl α-L-rhamnopyranoside esters with different chain length (C2-C18) were employed for PASS predication, antiviral, and *in vitro* antimicrobial activity test. The *in vitro* antimicrobial tests against four bacterial, and four fungal pathogens along with PASS predication indicated that these sugar esters acted as better antifungals as compared to antibacterial functionality. The study revealed that the incorporation of octanoyl (C8) and lauroyl (C12) group(s) at C-3 position of rhamnopyranoside possessed promising antimicrobial, and anti-carcinogenic potentiality with good pharmacokinetic (pkCSM), and drug likeness properties. Also, attachment of multiple ester groups enhanced various drug likeness, and medicinal chemistry friendliness conditions. Overall, the present findings might be useful for the development of rhamnopyranoside based novel MDR antimicrobial drugs.

Keywords: Benzyl α-L-rhamnopyranoside; Sugar esters (SEs); PASS predication; Antiviral; Antimicrobial activities; ADMET.

1. Introduction

Antimicrobial infections causes serious health problem worldwide. In this respect many natural and synthetic antibacterial drugs (penicillin, daptomycin, tigecycline, linezolid etc.), and antifungal drugs (amphotericin B, nystatin, flucytosine, papulacandins, azoles, echinocandin etc.) are in clinical use.[1] Different carbohydrate (sugar) residues are found in many naturally occurring different antimicrobial drugs like papulacandins, mannosylated nystatin (nystatin A1), and polyenes.[2] Generally, addition of sugar moieties in these drugs (nystatin A1, and amphotericin B) led to higher solubility and reduced hemolytic toxicity although biological activities of these polyene antifungals depend on the number and type of extended second sugar residue.[3]

Due to the highly specific interactions with physiological receptors carbohydrates participate in many crucial biological processes. Their esters, especially monosaccharide based sugar esters (SEs), are involved in many diverse biological events in organisms from all kind of life.[4] They are generally biodegradable, non-toxic, non-allergic, and non-irritating as are composed of a hydrophilic carbohydrate moiety, and one or more fatty acids as lipophilic moieties.[5] SEs have attracted considerable research interest and wide range of application in industry, and medicine mainly due to their considerable insecticidal, and antimicrobial activities.[6-7] Of the SEs, rhamnopyranoside derived esters are found to be of great importance as antigenic, anticarcinogenic,[8] antimicrobial,[9-10] and pharmacological properties. For example, 2-*O*-acetyl-3,4-di-*O*-(*E*)-*p*-methoxycinnamoyl-α-L-rhamnopyranoside(s) (**1**; Figure 1) isolated from *S. buergeriana* exerted significant protective effects against glutamate-induced neurodegeneration in primary cultures of cortical neurons.[11] Very recently, four new rhamnopyranose esters (e.g. **2a-d**) were isolated from the stem of *P. odorata* and found to be good scaffolds for developing new anti-breast cancer and antioxidant compounds.[12] All the observations indicated that esterification of the rhamnose moiety in these natural products is essential for high affinity binding and selectivity as well as for the development of anticancer agents (RSK inhibitors).[8,12]

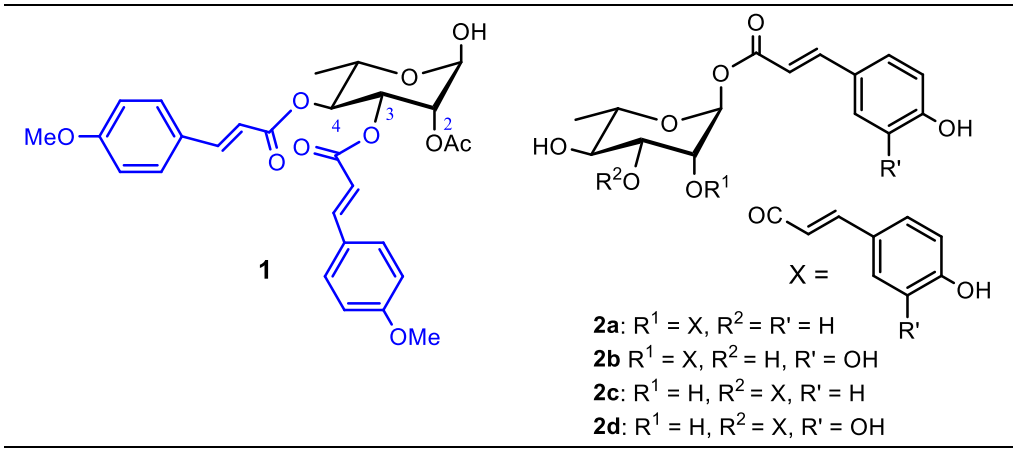


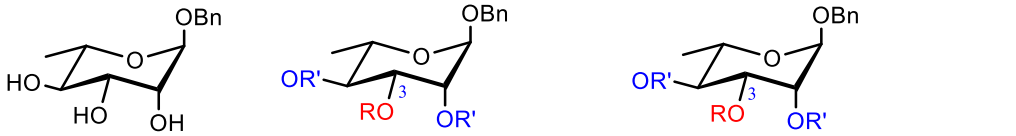
Figure 1. Naturally found bioactive rhamnopyranoside esters **1** and **2a-d**.

Emergence of multiple drug resistant (MDR) pathogenic bacteria and fungi is a global concern in addition to numerous obstacles encountered on delivery to the site of microbial infection. To combat with these MDR organisms there is urgent need for establishment of new chemotherapeutic agents with novel mode of action. In this respect as well as our continuous effort to find novel antimicrobial SEs,[13-16] we report herein the antimicrobial efficacy of several rhamnopyranoside esters against four pathogenic bacterial and four fungal strains along with ADMET.

2. Materials and methods

2.1. Materials: rhamnopyranoside esters 4-20

In the present study, seventeen esters with different side chain length and different groups **4-20** of benzyl α-L-rhamnopyranoside (**3**) were used as test chemicals. These compounds (mainly 3-*O*-acyl esters), as shown in Figure 2, were synthesized from **3** by dibutyltin oxide method followed by direct method and well characterized by Kabir and Matin.[17-18] For better comparison, standard antibacterial antibiotic tetracycline and antifungal antibiotic fluconazole were used.



3

4: R = Ac, R' = H
5: R = $C_7H_{15}CO$, R' = H
6: R = $C_9H_{19}CO$, R' = H
7: R = $C_{11}H_{23}CO$, R' = H
8: R = $C_{11}H_{23}CO$, R' = Ac
9: R = $C_{13}H_{27}CO$, R' = H
10: R = $C_{13}H_{27}CO$, R' = Ac
11: R = $C_{15}H_{31}CO$, R' = H
12: R = $C_{15}H_{31}CO$, R' = Ac

13: R = $C_{17}H_{35}CO$, R' = H
14: R = $C_{17}H_{35}CO$, R' = Ac
15: R = CH_3SO_2, R' = H
16: R = CH_3SO_2, R' = Ac
17: R = 4-$CH_3C_6H_4SO_2$, R' = H
18: R = 4-$CH_3C_6H_4SO_2$, R' = Ac
19: R = C_6H_5CO, R' = H
20: R = C_6H_5CO, R' = H

Figure 2. Rhamnopyranoside based SEs **4-20** as antimicrobials.

2.2. PASS predication

Initially, structures of the compounds were drawn and then converted into their SD file format and used to predict biological spectrum using PASS online version. Web based PASS (prediction of activity spectra for substances; http://www.pharmaexpert.ru/PASSonline/index.php) was used for the prediction of biological spectrum of these SEs.[9] This program is designed to anticipate a plethora of biological activities with 90% accuracy. PASS result is designated as Pa (probability for active compound) and Pi (probability for inactive compound). Only activities with Pa>Pi are considered as possible for a particular compound. Being probabilities, the Pa and Pi values vary from 0.000 to 1.000 and in general, Pa+Pi≠1, since these probabilities are calculated independently. The PASS prediction results were interpreted and used in a flexible manner, such as (i) when Pa>0.7, the chance to find the activity experimentally is high, (ii) if 0.5<Pa<0.7, the chance to find the activity experimentally is less, but the compound is probably not so similar to known pharmaceutical agents, and (iii) if Pa<0.5, the chance to find the activity experimentally is less, but the chance to find a structurally. Hence, the predicted activity

of spectrum is known as the intrinsic property of the compound.

2.3. Antiviral activities evaluation

Antiviral compounds (AVCs) are a special category of antimicrobial drugs used for treating viral infections by inhibiting the development of the viral pathogen(s) inside the host cell. For antiviral activity predication, we used online software (http://crdd.osdd.net/servers/avcpred) which showed percentage of inhibition.[19] SD file format of the compounds were used for predications. The evaluation conducted for the antiviral therapeutics development and also in identifying the best inhibitory compounds for further research.

2.4. *In vitro* antimicrobial activities evaluation

Two Gram-positive bacteria (*Bacillus cereus* BTCC 19, *Bacillus megaterium* ATCC 6633) and two Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* CRL, ICDDR,B*)* were used for *in vitro* antibacterial activity test using the disc diffusion method.[20-22] Dimethylformamide (DMF) was used as a solvent, and control for the test chemicals, and a 2% solution of each compound was used. The plates were incubated at 37 °C for 48 h. Mueller-Hinton (agar and broth) medium was used to culture the bacteria. Paper disc of 4 mm in a diameter and petri plate of 70 mm in a diameter was used throughout the experiment. These paper discs were sterilized in an autoclave and dried at 150 ºC in an oven. Then the discs were socked with test chemicals at the rate of 100 µg (dry weight) per disc for antibacterial analysis. Firstly, the plates were kept for 4 h at low temperature (4 ºC) and the test chemicals diffused from disc to the surrounding medium by this time. The plates were then incubated at (35±2) ºC for growth of test organisms and were observed at 24 h intervals for two days. The activity was expressed in terms of inhibition zone diameter in mm and each experiment was carried out three times. Tetracycline (antibiotic used for bacterial infections, brand name Tetrax-500, from Square Pharmaceuticals Ltd., Bangladesh) was used as a positive control and compared with tested chemicals under identical conditions.

The *in vitro* antifungal efficacy was investigated based on food poisoning technique[23-24] against four human pathogenic fungi viz. *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus* and *Penicillium notatum*. Sabouraud (agar and broth, PDA) medium was used for culture of fungi. Necessary amount of medium was taken in a conical flask separately and was sterilized in autoclave (at 121 ºC and 15 psi) for 15 minutes. After autoclaving, weighed amount of test chemical (2%) was added to the sterilized medium in conical flask at the point of pouring to obtain desired concentration. The flask was shaken thoroughly to mix the chemical with the medium homogeneously before pouring. The medium with definite concentration (2%) of chemical was poured at the rate of 100 µL in sterilized glass petri dishes individually, proper control was maintained separately and with sterilized PDA medium without chemical and three replications were prepared for each treatment. Linear mycelial growth of fungus was measured after 2~4 days of incubation and measured as percentage of zone of inhibition. The antifungal results were compared with that of standard antibiotic, fluconazole (brand name Omastin, 50 mg, Beximco Pharmaceuticals ltd., Bangladesh).

2.5. ADME/T analysis

In silico screening approaches using the pkCSM signatures were successfully used across five main different pharmacokinetic property classes to develop predictive regression and classification models.[25] This

prediction of drug could avoid the tremendous cost and time associated with the *in vivo* experiments, and attracted more and more attention. ADMET (absorption, distribution, metabolism, excretion and toxicity) analyses were conducted by using computational approaches. At first all the structures of rhamnopyranoside esters were drawn in ChemDraw 18.0 to collect InChI Key, isomeric SMILES and SD file format. ADMET of all the SEs were predicted by using pkCSM-pharmacokinetics[25] (http://biosig.unimelb.edu.au) and SwissADME[26] free web tools (http://www.swissadme.ch). SMILES (simplified molecular-input line-entry system) strings were used throughout the process.

3. Results

3.1. Biological activities using PASS

Initially, we applied prediction of activity spectra for substances (PASS) for activity test of the SEs **4**-**20**. PASS results in its designated as Pa and Pi form are presented in Table 1.

Table 1. Predicted biological activity of synthesized SEs **4**-**20** using PASS software.

Drug	**Biological activity**							
	Antibacterial		Antifungal		Anti-carcinogenic		Antioxidant	
	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi
3	0.561	0.011	0.654	0.013	0.680	0.009	0.607	0.004
4	0.617	0.008	0.700	0.010	0.770	0.006	0.541	0.005
5	0.604	0.009	0.728	0.008	0.737	0.007	0.491	0.007
6	0.604	0.009	0.728	0.008	0.737	0.007	0.491	0.007
7	0.604	0.009	0.728	0.008	0.737	0.007	0.491	0.007
8	0.608	0.008	0.714	0.009	0.605	0.012	0.438	0.009
9	0.604	0.009	0.728	0.008	0.737	0.007	0.491	0.007
10	0.608	0.008	0.714	0.009	0.605	0.012	0.438	0.009
11	0.604	0.009	0.728	0.008	0.737	0.007	0.491	0.007
12	0.608	0.008	0.714	0.009	0.605	0.012	0.438	0.009
13	0.604	0.009	0.728	0.008	0.737	0.007	0.491	0.007
14	0.608	0.008	0.714	0.009	0.605	0.012	0.438	0.009
15	0.492	0.017	0.584	0.020	0.566	0.014	0.431	0.10
16	0.525	0.014	0.600	0.018	0.496	0.020	0.362	0.016
17	0.464	0.20	0.548	0.024	0.394	0.032	0.407	0.011
18	0.497	0.017	0.568	0.022	0.345	0.043	0.337	0.018
19	0.588	0.009	0.662	0.012	0.740	0.007	0.559	0.005
20	0.608	0.008	0.670	0.012	0.661	0.010	0.479	0.007
TC	0.694	0.005	0.523	0.023	-	-	-	-
FZ	-	-	0.726	0.008	-	-	-	-

Pa = Probability 'to be active'; Pi = Probability 'to be inactive'; TC = tetracycline; FZ = fluconazole.

PASS predication (Table 1) of the rhamnopyranoside esters **4**-**20** were found 0.46<Pa<0.61 in antibacterial and 0.54<Pa<0.73 in antifungal. This clearly indicated that the SEs were more potent against fungi as compared to that of bacterial pathogens. Also, antifungal potentiality of some rhamnose fatty acyl esters were almost similar to fluconazole (Pa 0.73). We have extended our studies for anti-carcinogenic and antioxidant evaluation. Thus, PASS predication indicated 0.34<Pa<0.77 for anti-carcinogenic and 0.33<Pa<0.56 for antioxidant, which refers that the rhamnopyranoside esters were more potent

as anti-carcinogenic agents than that of antioxidant properties.

3.2. Antiviral activities

Having notable antifungal and anti-carcinogenic potentiality we were interested to predict antiviral activities of these esters **4-20**, and compared with Retrovir (antiviral drug), and Remdesivir (drug used for COVID-19) using antiviral software (Table 2).[19]

Table 2. Predicted antiviral activities of **3-20**, Retrovir and Remdesivir (% inhibition).

Drug	General	HBV	HCV	HHV	HIV
3	11.36	15.981	13.144	27.622	59.22
4	48.361	20.732	37.969	45.392	60.787
5	52.074	20.628	27.065	84.863	47.667
6	24.72	20.275	33.073	23.64	58.288
7	18.812	23.745	32.94	27.319	58.536
8	36.419	14.932	42.448	49.543	62.861
9	45.575	20.661	36.239	52.474	56.672
10	38.724	20.648	77.793	57.005	50.88
11	35.969	21.431	37.537	31.147	47.863
12	23.465	20.27	65.378	48.497	56.071
13	35.969	21.47	37.537	31.189	47.388
14	1.683	19.454	71.354	54.619	54.256
15	14.885	19.991	14.214	26.881	56.82
16	48.25	23.269	8.007	51.467	54.203
17	25.189	23.842	11.166	5.468	50.984
18	31.242	25.386	23.093	40.456	58.41
19	29.895	14.132	53.31	27.43	59.293
20	30.376	19.393	51.579	82.153	40.538
Retrovir	86.484	19.619	24.962	28.728	92.855
Remdesivir	55.91	13244	57.744	34.473	63.377

HBV = Hepatitis B virus; HCV = Hepatitis C virus; HHV = Human herpesvirus; HIV = Human immunodeficiency virus.

3.3. *In vitro* antimicrobial evaluation

3.3.1. Effects against bacteria

We followed disc diffusion method[20-22] for antibacterial efficacy test against two Gram-positive, and two Gram-negative organisms. The results of diameter of inhibition zone (mm) of the synthesized mannopyranoside type SEs against bacteria are presented in Table 3. It was found that these esters were moderate inhibitor against bacterial pathogens.

Table 3. Inhibition against bacterial pathogens by rhamnose esters.

Drug	Diameter of zone of inhibition in mm (100 µg dw / disc)			
	B. cereus	*B. megaterium*	*E. coli*	*P. aeruginosa*
3	NI	NI	NI	NI
4	NI	NI	11.0±0.24	NI
5	10.2±0.28	*17.4±0.44	*20.4±0.41	13.6±0.24
6	9.2±0.28	8.4±0.33	12.3±0.58	NI
7	10.6±0.33	*15.7±0.58	*19.3±0.59	NI
8	NI	14.8±0.38	14.8±0.83	NI
9	9.5±0.50	13.7±0.71	8.8±0.58	9.7±0.48
10	11.5±0.50	13.0±0.33	NI	NI
11	14.0±0.5	NI	NI	NI
12	14.7±0.64	NI	NI	NI
13	11.2±0.33	14.2±0.5	NI	NI
14	11.5±0.29	8.8±0.33	9.8±0.44	NI
15	NI	8.1±0.44	NI	NI
16	NI	10.5±0.50	NI	NI
17	13.8±0.91	12.9±0.50	NI	NI
18	14.1±0.33	13.3±0.5	NI	NI
19	NI	13.2±0.59	NI	9.1±0.33
20	8.0±0.24	14.3±0.33	NI	NI
****TC**	*21.0±0.51	*26.1±0.64	*25.0±0.58	*22.2±0.24

Data are presented as (Mean±SD). Values are represented for the triplicate of all the experiments. Significantly inhibition values are marked with * sign and, ** sign for reference antibiotic tetracycline (TC; 25 µg/disc). dw = Dry weight; NI = No inhibition. NI was observed for control DMF.

3.3.2. Effects against fungal pathogens

The effect of the rhamnose esters **4-20** against four pathogenic fungi[23-24] are presented in Table 4. The results, as presented in percentage inhibitions of mycelial growth, indicated that 3-*O*-acyl esters of rhamnopyranosides were very prone against tested fungal pathogens.

Table 4. Inhibition against fungal pathogens by **4**-**20**.

Drug	% Inhibition of fungal mycelial growth (100 µg dw / mL PDA)			
	A. flavus	*A. niger*	*A. fumigatus*	*P. notatum*
3	NI	NI	25.3±0.28	24.3±0.24
4	35.0±0.24	37.9±0.33	48.1±0.33	45.5±0.50
5	41.2±0.64	*67.3±0.59	*63.8±0.29	46.1±0.44
6	36.3±0.66	54.6±0.59	56.2±0.74	41.3±0.59
7	39.0±0.24	*61.7±0.71	59.3±0.68	48.2±0.50
8	48.8±0.59	*64.2±0.64	*68.0±0.50	54.3±0.50
9	43.7±0.44	35.2±0.84	49.0±0.33	45.5±0.64
10	54.3±0.48	55.8±0.28	59.0±0.50	38.5±0.50
11	42.9±0.84	38.0±0.50	52.0±0.84	33.0±0.74
12	44.0±0.59	44.0±0.50	55.0±0.50	28.9±0.59
13	NI	39.0±0.44	51.8±0.33	NI
14	27.1±0.64	41.6±0.33	49.0±0.33	48.0±0.50
15	NI	30.5±0.50	NI	NI
16	29.0±0.24	32.0±0.50	36.0±0.33	37.5±0.50
17	NI	26.4±0.44	NI	41.0±0.50
18	33.0±0.59	29.0±0.59	31.1±0.59	39.3±0.71
19	31.4±0.44	49.0±0.50	53.0±0.50	48.0±0.50
20	NI	33.0±0.44	*64.0±0.84	51.9±0.59
****FZ**	47.0±0.25	37.1±0.29	*62.0±0.58	*65.7±0.61

Data are presented as (Mean±SD). * = Significant inhibition; ** = Reference antibiotic fluconazole (FZ, 50 µg/mL PDA). dw = Dry weight; NI = No inhibition; NI was observed for control DMF.

3.4. ADME/T analysis

Absorption, distribution, metabolism, excretion, and toxicity (ADMET) constitute the pharmacokinetic profile of a drug molecule and play key roles in the discovery/development of drugs, pesticides and related fields. ADMET properties, as derived from pkCSM-pharmacokinetics,[25] reveal that (Table 5) most of the rhamnose derived sugar esters (**5**,**6**,**8**,**10**,**12**,**16**-**20**) showed good to excellent absorption value. Distribution through BBB and CNS permeability indicated that these esters are comparable to that of fluconazole.

SwissADME was used for drug likeness calculation and summarized in Table 6. We observed that all the SEs have good hydrogen bonds donor and acceptor which is in consistent with the Lipnski's rule of five.[27]

Table 5. Admet calculation of rhamnose derived SEs **4**-**20**.

Drug	Absorption			Distribution		Metabolism	Excretion	Toxicity	
	C2P	HIA (%)	P-gpI	BBB (permeability)	CNS	CYP3A4 substrate	Total clearance	hERG inhibitor	Toxicity (LD_{50})
3	0.055	58.714	No	-0.942	-3.156	No	1.498	No	2.498
4	0.276	68.272	No	-0.391	-3.456	No	1.483	No	2.474
5	1.13	92.485	Yes	-0.298	-3.153	Yes	1.664	No	2.952
6	1.084	91.798	Yes	-0.308	-3.114	Yes	1.719	No	3.221
7	0.778	91.111	Yes	-0.317	-3.091	Yes	1.775	No	3.484
8	0.96	82.659	Yes	-1.316	-3.043	Yes	1.43	No	2.605
9	0.701	90.424	Yes	-0.326	-3.068	Yes	1.566	No	3.723
10	0.936	84.11	Yes	-1.359	-3.02	Yes	1.46	No	2.469
11	0.624	89.736	Yes	-0.336	-3.044	Yes	1.598	No	3.918
12	0.912	85.561	Yes	-1.402	-2.996	Yes	1.491	No	2.351
13	0.547	89.049	Yes	-0.345	-3.021	Yes	1.631	No	4.04
14	0.888	87.011	Yes	-1.445	-2.973	Yes	1.521	No	2.246
15	0.618	54.959	No	-0.763	-3.685	No	1.545	No	2.269
16	0.941	61.744	No	-1.505	-3.655	Yes	1.496	No	2.319
17	1.143	74.098	Yes	-0.54	-3.444	Yes	1.42	No	2.151
18	1.061	80.638	Yes	-1.526	-3.467	Yes	1.471	No	2.191
19	1.328	94.422	Yes	-0.142	-3.28	Yes	1.431	No	2.095
20	1.162	89.087	Yes	-1.107	-3.302	Yes	1.506	No	2.885
FZ	1.191	87.821	No	-1.2	-3.221	No	0.386	No	2.21

C2P = Caco-2 permeability (log Papp in 10^{-6} cm/s, >0.90 indicates high permeability); HIA = Human intestinal absorption (% absorbed, >30% is better absorbed); P-gpI = P-glycoprotein inhibitor; BBB is expressed in logBB (logBB >-1.0 is moderately cross blood brain barrier); CNS is expressed as logPS (logPS>-2.0 can easily penetrate the CNS); Total clearance is expressed in log mL/min/kg; Toxicity is calculated in oral rat acute toxicity (mol/kg); FZ = fluconazole.

Table 6. Calculation drug likeness using SwissADME.

Drug	HB acceptors	HB donors	TPSA Å²	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	PAINS alerts
3	5	3	79.15	No	No	No	No	No	0
4	6	2	85.22	No	No	N o	No	No	0
5	6	2	85.22	No	No	No	Yes	Yes	0
6	6	2	85.22	No	No	No	Yes	Yes	0
7	6	2	85.22	No	No	No	Yes	Yes	0
8	8	0	97.36	No	No	Yes	No	Yes	0
9	6	2	85.22	No	No	No	Yes	Yes	0
10	8	0	97.36	No	No	Yes	No	Yes	0
11	6	2	85.22	No	No	No	Yes	Yes	0
12	8	0	97.36	No	No	No	No	Yes	0
13	6	2	85.22	No	No	No	No	Yes	0
14	8	0	97.36	No	No	No	No	Yes	0
15	7	2	110.67	No	No	No	No	No	0
16	9	0	122.81	No	No	No	No	No	0
17	7	2	110.67	No	No	No	No	Yes	0
18	9	0	122.81	No	No	No	Yes	Yes	0
19	6	2	85.22	No	No	No	Yes	Yes	0
20	8	0	97.36	No	No	Yes	No	Yes	0

*HB = Hydrogen bond, TPSA = Topological polar surface area, PAINS = Pan-assay interference compounds

4. Discussion

4.1. PASS properties

Initially PASS predication (Table 1) results of **4-20** (0.46<Pa<0.61 for antibacterial and 0.54<Pa<0.73 for antifungal) indicated that the SEs were more potent against fungi as compared to that of bacterial pathogens. Interestingly, anti-carcinogenic probability data of aliphatic sugar esters are in complete accord with the *in vivo* and *in vitro* results reported for compound **1** and **2a-d**.[8,11-12] The data also indicated that for both antifungal and anti-carcinogenic properties, the aliphatic rhamnopyranoside esters **4-14** were more promising than sulphonyl esters **15-18**.

4.2. Antiviral potentiality

We predicted antiviral potentiality of the sugar esters **4-20** (Table 2) using online software. It was observed that the introduction of ester groups moderately increased activity of SEs against HIV. To our surprise, octanoyl compound **5** (84.864%) and benzoyl compound **20** (82.153%) were found significantly potential against human herpesvirus as compared to Retrovir (28.728%) and Remdesivir (34.473%). Also, myristoyl compound **10** (77.793%) and stearoyl compound **14** (71.354%) were more prone against hepatitis C virus than Retrovir (24.962%) and Remdesivir (57.744%).

4.3. *In vitro* antimicrobial efficacy

In the next step, we evaluated antimicrobial activities of the SEs **4-20**. It was found (Table 3) that these esters were moderate inhibitor against bacterial pathogens. However, octanoate **5** and lauroate **7** were found to be better active against *E. coli* and *B. megaterium*. In general, rhamnose 3-*O*-esters were more susceptible against Gram-positive pathogens as compared to Gram-negative organisms (Table 3). On the other hand, the rhamnose esters **4-20** were found very prone against tested fungal pathogens (Table 4). SEs showed better inhibition zone against *A. niger*, and *A. fumigatus*. Octanoate **5**, and lauroate **8** were found more potential even better than that of the fluconazole. Thus, most of the rhamnopyranoside based SEs **4**–**20** possess excellent potentiality towards all tested fungal pathogens than the bacterial organisms. Interestingly, these *in vitro* results are in complete agreement with PASS predication results (Table 1).

Cytochromes P450's (CYP450) are responsible for metabolism of many drugs. Its inhibitors dramatically alter pharmacokinetic properties and hence we checked the metabolism property by calculating CYP3A4 substrate activity. Most of the hydrophobic esters (except **4** and **15**) could be metabolized by CYP450 although standard antifungal was found to possess non-metabolic property. As the SEs are metabolized they can pass through kidney as observed from total clearance value (Table 5).

4.4. ADME/T properties

As these SEs showed excellent antifungal activities, we scanned their ADMET properties and presented in Table 5. Rhamnopyranoside esters **4-20** showed non-inhibitory properties against hERG (human ether-a-go-go gene) indicating their nontoxic drug nature (Table 5). Inhibition of the potassium channels by hERG mainly generate QT syndrome- leading to fatal ventricular arrhythmia, and hence need to withdraw many drugs from pharmaceutical market.[25] Also, it is important to consider the lethal dosage (LD_{50}) values and is used to assess the relative toxicity of different molecules.[25] Here most of the SEs LD_{50} values (~3 mol/kg) are in good consistent with the value of fluconazole (2.21 mol/kg).

SwissADME of the SEs, as presented in the Table 6, indicated that topological polar surface area (TPSA) of the compounds possess good polarity, where the TPSA value should be less than 140 $Å^2$, more the

value more the polarity. The total polar surface area in these molecules was in excellent agreement with the most important rules of drug likeness.[27] The CYP enzymes, particularly isoforms 1A2, 2C9, 2C19, 2D6, and 3A4, are responsible for about 90% oxidative metabolic reactions. Inhibition of CYP enzymes will lead to inductive or inhibitory failure of drug metabolism. Pan-assay interference compounds (PAINS) are chemical compounds that often give false positive results in high-throughput screens. PAINS tend to react nonspecifically with numerous biological targets rather than specifically affecting one desired target. Here PAINS revealed no violation with these rhamnose esters.

The most of the commercial antifungal drugs (fluconazole; itraconazole; voriconazole; terbinafine) are designed to deter the activity of CYP51A1 enzyme to synthesize essential ergosterol. On the basis of our study, it could be thought that the SEs restricted CYP51 enzyme activity by binding with it, and hence ultimately fungal ergosterol couldn't form. The unavailability of ergosterol ultimately causes disruption of the fungal cell wall.

5. Conclusion

Natural and synthetic antimicrobial drugs are clinically useful for the treatment of simple to life threatening infections. However, toxicity and evolution of antimicrobial drug resistance is an almost inevitable process in the microbial world and hence novel antimicrobial drugs preferably with novel mode of action, improved efficacy as well as reduced side-effects are essential. Thus, a series of 3-*O*-acyl esters of rhamnopyranosides were screened for biological activities successfully. Both PASS and *in vitro* analyses established them as promising antifungal and anti-carcinogenic agents. Especially rhamnopyranoside esters with octanoyl (C8) and lauroyl (C12) chains, as in compound **5** and **8**, were found promising drug candidates comparable to antifungal antibiotic fluconazole. ADMET properties revealed that incorporation of multiple ester groups enhances various drug likeness, and medicinal chemistry friendliness conditions. More related studies are in progress in our laboratory and will be disclosed soon.

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Conflict of interest

Authors declare no conflict of interests.

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