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Drug-likeness and antimicrobial activity of 5-(4-bromophenyl)-3-{3-methyl-4-[(4-substitutedbenzyl)oxy]phenyl}-4,5-dihydro-1,2-oxazole

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ABSTRACT

This paper reports the drug-likeness and antimicrobial activity of 5-(4-bromophenyl)-3-{3-methyl-4-[(4-substitutedbenzyl) oxy] phenyl}-4, 5-dihydro-1, 2-oxazole (5a-g). The drug-likeness was predicted computationally whereas antimicrobial activity was assessed against selected strains of bacteria and fungi following the measurements of inhibition zones and minimum inhibitory concentrations. The tested compounds exhibited oral bioavailability and followed the Lipinski's 'rule of five'. The results revealed that the compounds 5a, 5b, 5f and 5g had good antimicrobial activity against almost all the bacterial & fungal strains tested. Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of some of the compounds (5f, 5g) were found to be several folds higher than their corresponding MIC values. Compounds (5f, 5g) bearing electron withdrawing and donating functional groups were found out to be most active among others. Interestingly, the compounds which showed maximum antimicrobial activity also have higher drug-likening score.

Keywords: Drug-likeness; Computational designing, Lipinski's rule; Isoxazole; Antibacterial, Antifungal.

INTRODUCTION

Microbial diseases are now more common and being still difficult to diagnose clinically. During the last two decades, several classes of antimicrobial agents have been discovered [1-4]. Infectious diseases, which are known to be the second leading cause of deaths, are rapidly growing during the recent past due to multiple microbial resistances [5-7]. Increasing multidrug-resistance, prevalence of bacterial strains and reduced susceptibility to antibiotics prompts the need to discover new antimicrobial agents [8]. In this context, organic compounds bearing isoxazoline moiety have been found to possess potent antibacterial and antifungal activities [9-11].

It has been found that due to insufficient bioavailability many promising chemical entities and drug candidates failed to be further developed into effective drugs. Literature suggested that in the drug discovery and development process, about 95% of lead compounds failed, out of these 50% were due to critical liberation, absorption, distribution, metabolism, and excretion (LADME) properties [12, 13]. Prediction of oral bioavailability may be supervised by Lipinski's 'rule of five' using *in-silico* method, which could be used to predict the compounds absorption and permeability qualitatively [14]. Previously, we reported the synthesis and antimycobacterial activity of 4-[5-(substituted phenyl)-4, 5-dihydro-3-isoxazolyl]-2-methylphenols [15].The present paper reports the antimicrobial and drug-likening properties (using Molinspiration, 2008 and MolSoft, 2007 softwares) by selecting and exploring the most active compound i.e. lead compound (4l) of the same series (4a-m) in order to get better lead compounds for further research.

MATERIALS AND METHODS

Computation of drug-likeness properties

Drug-likeness is equilibrium amongst the molecular properties of a compound which directly affects pharmacodynamics and pharmacokinetics of a drug in human body [16]. A variety of pharmacophoric features including hydrophobicity, molecular dimension, and flexibility control the activities of molecules in a living system. During the last few years there are numerous models being proposed to relate structure-bioavailability relationship [17-19]. Possibly the best known of these studies is the 'rule-of-five' given by Lipinski [14]. Depending on these four molecular descriptors, the approach generates a vigilant about apparent absorption trouble if at least two of the following conditions are fulfilled: (1) calculated log P >5 (2) molecular weight >500; (3) total number of hydrogen-bond acceptors >10; (4) total number of hydrogen-bond donors >5. *Absorption, polar surface area, and ''rule of five" properties*

It is presumed that the flexibility of a molecule (generated through number of rotatable bonds), small polar surface area or number of hydrogen bond (donors and acceptors), play a vital role towards imparting an excellent oral bioavailability [20, 21]. Key drug or drug-like properties such as membrane permeability and bioavailability is always linked with some basic molecular descriptors viz: logP (partition coefficient), molecular weight (MW), or number of hydrogen bond acceptors and donors in a molecule [22]. Lipinski proficiently used these molecular properties to generate his ''rule of five" which is frequently used for the screening of drug-like properties. Table1 contains percentage of absorption (%ABS), polar surface area (PSA) and other Lipinski parameters of the compounds under investigation (5a-g). Amount of absorption is defined by the percentage of absorption and calculated by using the formula: %ABS =109 - 0.345 PSA [23]. Polar surface area (PSA) was calculated by the fragment-based method [24, 25]. The series (5a-g) under investigation has all the compounds with number of hydrogen bond donors and acceptors were found within the range as suggested by Lipinski as shown in table 1.

Number of rotatable bonds is essential to observe the effect of conformational changes of molecules during binding with receptors or channels. Literature suggested that for a good oral bioavailability, number of rotatable bond should be ≤10 [20]. The test compounds of present series (5a-g) in general have low number of rotatable bonds (3–7) and hence, reveal small conformational flexibility.

Table-1: Calculated absorption (%ABS), polar surface area (PSA), Lipinski parameters and drug-likeness model score of the compounds (5a-g) investigated

Compounds	y	% ABS	Volume(A3)	PSA (A2)	NRO TB	HBA	HBD	LogP Calcd.	Formula Weight	Drug-likeness Model score
5a	H	98.69	365.26	29.87	5	3	0	6.91	421	0.05
5b	Cl	98.69	382.45	29.87	5	3	0	6.90	455	0.14
5c	Br	98.69	387.12	29.87	5	3	0	7.04	498	0.03
5d	F	98.69	371.18	29.87	5	3	0	6.46	439	0.01
5e	CF_3	98.36	390.22	30.83	7	3	0	7.45	504	0.04
5f	OH	92.61	375.81	47.49	5	4	1	5.93	437	1.28
5g	CH_3	98.36	358.68	30.83	5	3	0	6.80	436	1.11
Std(Ciprofloxacin)	-	78.58	373.70	62.10	3	4	2	1.09	333	1.36
Std(Voriconazole)	-	78.60	298.05	62.05	5	5	1	1.60	349	0.98

Polar surface area (PSA) of a molecule is another valuable parameter for drug transport across the membrane. PSA is amount of plane of polar atoms (oxygen, nitrogen and attached hydrogen) in a drug molecule. All the compounds in this series have more than 90% absorption (table1).

Drug-likeness model score is a numerical value and it is controlled by the mutual effect of physicochemical as well as pharmacokinetics and pharmacodynamics properties of a molecules. It has been stated that the tested compound having zero or negative score cannot be selected as drug like [14]. In the present experiment, maximum drug-likeness score were obtained in the order of 5f > 5g > 5b > 5a > 5e > 5c > 5d. Only two compounds have drug-likeness score closer to the standard drugs, Ciprofloxacin (1.36) and Voriconazole (0.98) used in this experiment for antibacterial and antifungal activity, respectively. It is remarkable to see that the drug having maximum score has also shown pharmacological activity almost in the similar fashion.

Chemistry

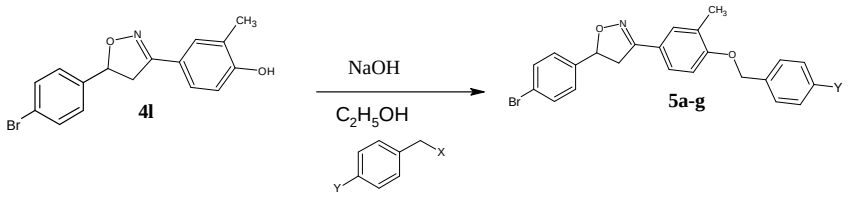
The present research work explores the drug likeness and antimicrobial potential of newly synthesized chemical compounds. The new scheme is outlined as 5a-g.

Lead compound of our earlier work was selected as a starting material for the present series (5a-g) under investigation. All the chemicals used in present research were purchased from Loba Chem(India) and Sigma Aldrich(USA). These chemicals were used in the experiments without any further purification.

The melting points of the compounds were determined using open tube capillary method and are uncorrected. The compounds purity was checked via development on commercial thin layer chromatography (TLC) plates using the mobile solvent system (*n*-hexane: EtOAc, 80: 20 v/v).

5-(4-bromophenyl)-3-{3-methyl-4-[(4-substitutedbenzyl) oxy] phenyl}-4, 5-dihydro-1,2-oxazole(5a-g) General procedure: A mixture of 0.01 moles of 4l was taken in absolute ethanol and stirred gently. Almost 10 pellets of sodium hydroxide paste were added to this solution and again stirred the reaction at 80^0C for 1h. Equiamolar quantity of substituted benzyl halides were added drop wise to the reaction mixture. After 2-3 h development (*n*-hexane: EtOAc, 80: 20 elution solvent v/v), TLC band was checked and found single spotted. The reaction mixture was transferred to the crushed ice and neutralized with HCl. The product so obtained was filtered and recrystallized with ethanol to yield desired compounds (5a-g).

Scheme-1



X= Cl, Br

Y= H, Cl, Br, F, CF_3, OH, CH_3

3-[4-(benzyloxy)-3-methylphenyl]-5-(4-bromophenyl)-4, 5-dihydro-1, 2-oxazole (5a)
M.F. $C_{23}H_{22}BrNO_2$;Yield (85%); m.p. 140-142 °C. IR :(KBr) cm^{-1}: 2907(OCH_2), 1622 (C=C), 1568 (C=N), ^{1}H NMR (DMSO-d6) ppm: 6.89-7.52 (8H, m, Ar), 5.92-6.12(1H, t, CH, J=6.6 Hz), 4.52(2H, s, OCH_2), 3.57-3.61(2H, dd, CH_2, J=6.4Hz), 2.30(3H, s, CH_3).

5-(4-bromophenyl)-3-{4-[(4-chlorobenzyl) oxy]-3-methylphenyl}-4, 5-dihydro-1, 2-oxazole (5b)
M.F. $C_{23}H_{21}BrClNO_2$;Yield (88%); m.p. 136-138 °C. IR :(KBr) cm^{-1}: 2905(OCH_2), 1632 (C=C), 1561 (C=N), ^{1}H NMR (DMSO-d6) ppm: 6.91-7.47 (8H, m, Ar), 6.11-6.14(1H, t, CH, J=6.6 Hz), 4.52(2H, s, OCH_2), 3.54-3.59(2H, dd, CH_2, J=6.4Hz), 2.33(3H, s, CH_3).

3-{4-[(4-bromobenzyl)oxy]-3-methylphenyl}-5-(4-bromophenyl)-4,5-dihydro-1,2-oxazole(5c)
M.F. $C_{23}H_{21}Br_2NO_2$;Yield (81%); m.p. 139-141 °C. IR :(KBr) cm^{-1}: 2907(OCH_2), 1628 (C=C), 1564 (C=N), ^{1}H NMR (DMSO-d6) ppm: 7.02-7.51 (8H, m, Ar), 5.78-5.91(1H, t, CH, J=6.2 Hz), 4.55(2H, s, OCH_2), 3.52-3.56(2H, dd, CH_2, J=6.5Hz), 2.34(3H, s, CH_3).

5-(4-bromophenyl)-3-{4-[(4-fluorobenzyl)oxy]-3-methylphenyl}-4,5-dihydro-1,2-oxazole(5d)
M.F. $C_{23}H_{21}BrFNO_2$;Yield (78%); m.p. 132-134 °C. IR :(KBr) cm^{-1}: 2904(OCH_2), 1633 (C=C), 1569 (C=N), ^{1}H NMR (DMSO-d6) ppm: 6.99-7.48 (8H, m, Ar), 5.89-5.96(1H, t, CH, J=6.6 Hz), 4.52(2H, s, OCH_2), 3.49-3.54(2H, dd, CH_2, J=6.6Hz), 2.29(3H, s, CH_3).

5-(4-bromophenyl)-3-(3-methyl-4-{[4-(trifluoromethyl)benzyl]oxy}phenyl)-4,5-dihydro-1,2-oxazole(5e)
M.F. $C_{24}H_{19}BrF_3NO_2$; Yield (88%); m.p. 141-142 °C. IR :(KBr) cm^{-1}: 2905(OCH_2), 1636 (C=C), 1571 (C=N), ^{1}H NMR (DMSO-d6) ppm: 6.96-7.55 (8H, m, Ar), 5.77-5.81(1H, t, CH, J=6.3 Hz), 4.52(2H, s, OCH_2), 3.42-3.51(2H, dd, CH_2, J=6.4Hz), 2.31(3H, s, CH_3).

4-({4-[5-(4-bromophenyl)-4,5-dihydro-1,2-oxazol-3-yl]-2-methylphenoxy}methyl)phenol(5f)
M.F. $C_{23}H_{20}BrNO_3$;Yield (79%); m.p. 137-139 °C. IR :(KBr) cm^{-1}: 2907(OCH_2), 1641 (C=C), 1568 (C=N), ^{1}H NMR (DMSO-d6) ppm: 7.10-7.52 (8H, m, Ar), 5.91-6.31(1H, t, CH, J=6.5 Hz), 4.52(2H, s, OCH_2), 3.56-3.61(2H, dd, CH_2, J=6.3Hz), 2.34(3H, s, CH_3).

5-(4-bromophenyl)-3-{3-methyl-4-[(4-methylbenzyl)oxy]phenyl}-4,5-dihydro-1,2-oxazole(5g)
M.F. $C_{24}H_{22}BrNO_2$;Yield (86%); m.p. 141-143 °C. IR :(KBr) cm^{-1}: 2907(OCH_2), 1640 (C=C), 1564 (C=N), ^{1}H NMR (DMSO-d6) ppm: 6.94-7.52 (8H, m, Ar), 5.98-6.14(1H, t, CH, J=6.6 Hz), 4.52(2H, s, OCH_2), 3.61-3.69(2H, dd, CH_2, J=6.4Hz), 2.33(3H, s, CH_3).

Pharmacology

Antibacterial studies

Compounds (5a-g) were evaluated for their antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus Subtilis* (recultured) bacterial strains by disc diffusion method [26, 27]. 1-2 x10^7 c.f.u./mL and 0.5 McFarland standards inoculums was introduced onto the surface of sterilized agar plates, and for equal distribution of the inoculum a sterile glass spreader was used. A disc having 6.25 mm in diameter were made from Whatman no.1 filter paper and then sterilized by heating at 140 ^oC for 1 h. The sterilized discs, earlier drenched with the compound under test in DMSO solution of known concentration (100 µg and 200 µg /disc), were carefully placed on the agar culture plates. The plates were incubated at 37 ^oC and after 24 h inhibition zones were measured in diameter. For 24 h at 37 ^oC all the plates were inverted and incubated properly. Ciprofloxacin was chosen in this particular experiment as a standard drug. Zones of inhibition were calculated and compared with the controls carefully. Zones of inhibition for antibacterial activity values are given in table 2.

Table- 2: Antibacterial activity of compounds (5a-g)

Compounds.	Concentration (µg/mL)	Zone of inhibition (mm)			
		S.aureus	*B.subtilis*	*E.coli*	*P.aeruginosa*
5a	100	14	12	17	19
	200	15	13	25	22
5b	100	15	13	19	19
	200	27	15	20	21
5c	100	13	12	16	19
	200	15	12	15	18
5d	100	12	10	13	16
	200	13	12	14	15
5e	100	11	10	13	19
	200	13	13	16	20
5f	100	22	19	28	29
	200	24	20	27	32
5g	100	19	15	20	23
	200	21	16	22	24
Std(Ciprofloxacin)	100	23	20	27	33
	200	24	22	28	34

Broth dilution technique was used for the assessment of minimum inhibitory concentrations (MICs). The nutrient broth containing two fold diluted amount of test compounds and controls were inoculated with roughly 5 x 10^5 c.f.u. of actively dividing bacterial cells. These cultures were again incubated for another 24 h at 37 ^oC and then growth of microbes was monitored visually as well as spectrophotometrically. Therefore, MIC is the lowest concentration (highest dilution) required to seize growth of bacteria.

Lowest bacterial concentration was obtained by transferring 0.1 mL volume from each tube and spread on agar plates. Following 18-24 h of incubation at 35 ^oC quantity of c.f.u. was counted. MIC and MBC concentrations are given in table 3.

Table- 3: MIC and MBC results of compounds (5a-g)

Compounds	*S.aureus*		*B.subtilis*		*E.coli*		*P.aeruginos*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
5a	6	12.5	6	12.5	12.5	25	12.5	25
5b	6	12.5	6	6	12.5	25	6	12.5
5c	6	12.5	6	6	6	12.5	6	12.5
5d	6	6	6	6	6	6	6	12.5
5e	6	12.5	6	12.5	6	6	6	12.5
5f	12.5	25	6	12.5	12.5	25	25	50
5g	6	12.5	12.5	25	6	12.5	6	12.5
Std(Ciprofloxacin)	6	12.5	6	12.5	6	12.5	6	12.5

Antifungal studies

Fungal strains *C. albicans* and *A. niger* in DMSO solvent were used for antifungal activity with agar diffusion technique [28, 29]. To make Sabourauds agar media a definite amount of peptone (1g), D-glucose (4g) and agar (2g) was dissolved in deionized water (100 mL) and adjusting pH 5.7. A suspension of spore of fungal strain was made for lawing and normal saline were also prepared separately To a 3 mL saline, a loopful of particular fungal strain was added to acquire a suspension of particular species. To each petri dish, 20 mL of agar media was poured. Excess suspension was transferred and the plates were placed in an incubator for drying at 37 ^oC for 1 h. Wells were made by means of an agar punch and each was labelled. A control was also arranged in triplicate and maintained at 37^oC

for 3-4 days. Voriconazole was used as a model drug (positive control) to compare fungal activity of each compound. Fungal zones of inhibition were calculated and compared with the controls and values are given in table 4.

Table- 4: Antifungal activity of compounds (5a-g)

Compounds.	Concentration (µg/mL)	Zone of inhibition (mm)	
		Candida Albicans	*Aspergillus Niger*
5a	100	15	15
	200	16	14
5b	100	18	17
	200	15	16
5c	100	15	11
	200	13	9
5d	100	17	14
	200	10	8
5e	100	9	6
	200	11	10
5f	100	25	19
	200	27	18
5g	100	22	16
	200	20	14
Std(Voriconazole)	100	28	25
	200	30	26

The nutrient broth containing two times diluted amount of test and controls was inoculated about $1.6x10^4$ – 6 x 10^4 c.f.u./mL All the chemicals used in present research were purchased from Loba Chem (India) and Sigma Aldrich(USA). These chemicals were used in the experiments without any subsequent purification.

Cultures growth was monitored by incubating at 35^{o}C for 48 h. The minimum inhibitory concentration (MIC) is defined as the smallest concentration (highest dilution) necessary to inhibit the growth of fungus. For the analysis of minimum fungicidal concentration (MFC), 0.1 mL volume was taken from each tube and spread on agar plates. Number of c.f.u. was counted after 48 h of incubation. MFC is the smallest drug concentration at which 99.9% of the inoculums were killed. Values of MIC and MFC are given in table 5.

Table- 5: MIC and MFC Results of Compounds (5a-g)

Compounds	*Candida Albicans*		*Aspergillus Niger*	
	MIC	MFC	MIC	MFC
5a	25	50	50	100
5b	12.5	12.5	25	50
5c	50	100	-	100
5d	50	-	-	-
5e	12.5	100	-	50
5f	25	25	12.5	25
5g	25	12.5	12.5	12.5
Std(Voriconazole)	6	12.5	6	12.5

RESULTS AND DISCUSSION

Chemistry

The5-(4-bromophenyl)-3-{3-methyl-4-[(4-substitutedbenzyl)oxy]phenyl}-4,5-dihydro-1,2-oxazole(5a-g) depicted in this study are presented in table 1 and outlined in scheme 1. We took 4l as starting material and explored the OH group present on phenyl ring. Starting material (4l) was directly reacted with substituted benzyl halides in highly basic condition in 2-3 h yielding 78-88% of the desired compounds (5a-g). Analytical and spectral data (NMR, IR) were found in full support of the titled compounds. In general FT-IR data indicates that the OCH_2 group vibrates at 2907 cm^{-1} which is characteristics for compounds. NMR data also proves the characteristics proton (OCH_2) appears at δ 4.5 which indicates the change of functional group from OH to OCH_2 of the compounds (5a-g) investigated. Moreover, other substituent's/groups are already present in starting material and appeared in respective final compounds shown in experimental section.

Pharmacology

Data revealed for antibacterial activity indicated that the majority of the synthesized compounds did not show good bacterial inhibition. Among these compounds, 5a, 5f and 5g exhibited good antibacterial activity particularly against *Escherichia coli* and *Pseudomonas aeruginosa*. Compound 5f and 5g showed almost comparable antibacterial activity as standard drug Ciprofloxacin. Minimum bactericidal concentration (MBC) of several compounds was two folds higher than their corresponding MIC results.

The data regarding antifungal screening showed that most of the compounds have moderate activity. Among screened compounds 5f and 5g showed good inhibition against fungal strains, *Candida albicans* and *Aspergillus niger*. Minimum fungicidal concentration (MFC) of compounds was several folds higher than their respective MIC values.

It was pleasant to see that most of the tested compounds were good to have oral bioavailability and followed the Lipinski ‘‘rule of five”. By investigating the tested compounds, it may be postulates that compound having electron withdrawing group (Br) and electron donating group (OH, CH_3) on two phenyl ring of same compound had great impact on antimicrobial activity as in 5f, and 5g. These functional groups might act as pharmacophoric descriptors. This result is also evidenced by the fact that those compounds having maximum drug-likeness score had also higher antimicrobial affinity among the series. It is possible to predict that further derivatization of such moiety might lead to achieve highly promising antimicrobial agents.

CONCLUSION

Compounds bearing electron withdrawing and donating functional groups showed good impact on microbial inhibition. Essentially, electron donating (OH) phenyl ring might act as a pharmacophoric descriptor in this case. It is interesting to reveal that the compounds having maximum drug-likeness score also had the highest antimicrobial susceptibility among the studied series. These results are very encouraging and provide novel lead compounds for future search of antimicrobial drugs.

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