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Synthesis, Characterization and Antimicrobial Activity of Some Novel Veratric Derivatives

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ABSTRACT

Novel bioactive molecules are designed by using molecular hybridization techniques which is based on the recognition of pharmacophoric sub-unities in the molecular structure of two or more known bioactive derivatives. Adequate fusion of these sub-unities, lead to the design of new hybrid architectures that maintain pre-selected characteristics of the original templates. All the designed molecules were than synthesized and evaluated for their antimicrobial activity.

Introduction

In developing countries, the resources available for the control of any disease is limited, therefore development of inexpensive but effective, acceptable, but nontoxic drug regimens for mass treatment of patients is of great importance. Infectious diseases remain the largest cause of death in the world today, greater than cardiovascular disease or cancer. Focusing on cures for infectious diseases like tuberculosis, malaria in emerging and third world countries who requires immediate attention as these products would not be viable for global MNCs to develop in view of their low pricing requirements. Anti infective agents are substances capable of acting against these infection, by inhibiting the spread of an infectious agent or by killing the infectious agent outright. Anti-infective is a general term that encompasses agents which could be antibacterials, antibiotics, antimycobacterials, antifungals, antiprotozoans or antivirals. Development of new antibacterial agents against several microorganisms, such as Bacillus anthracis, Yersinia pestis, and Francisella tularensis that can be used as biological weapons of mass destruction by terrorists, also need urgent attention.[1]

Unfortunately, the widespread emergence of resistance to antibiotics in pathogenic bacteria over the past 30 years is now a serious threat to global public health and could undermine the major advances achieved in the treatment of infection.[4-10] The increasing incidence of multidrug resistance among bacterial pathogens represents one of the major challenges and thus leads to another reason for enhanced requirement for the development of potent antiinfective agent.

Present work is based on rational designing of novel bioactive molecules by using molecular hybridization techniques. It is based on the recognition of pharmacophoric sub-unities in the molecular structure of two or more known bioactive derivatives which, through the adequate fusion of these sub-unities, lead to the design of new hybrid architectures that maintain pre-selected characteristics of the original templates.[15] All the synthesized veratric acid derivatives were screened for their antimicrobial activity against bacterial strains by utilising microtitre-plate used for determination of minimum inhibitory concentration (MIC) values.[57]

Experimental work

Design of novel Antimicrobial Molecules using Molecular Hybridization technique: The treatment of infectious diseases still remains a real perceived need for the discovery of new compounds endowed with antimicrobial activity, which are distinct from those of well-known classes of antibacterial agents to which many clinically relevant pathogens are now resistant.

The principle for designing of new derivatives was based on molecular hybridization approach by taking into consideration the role of the veratric acid nucleus as important biophore (scaffold) and subunit hydrazone moiety (NHN=CHR), since some recently reported derivatives with this chemotype have demonstrated interesting anti-infective activity. It also taken into account the fact that the N-acylhydrazone moiety (NAH, RCONHN=CHR) show an aza-vinylogue relationship with the amide group present in peptides, as the primary sites of hydrolysis catalyzed by proteases, and that iminic double bond can work like an hydrophobic anchor.

Based on the observations of two hybridized scaffolds incorporating 3,4-dimethoxy benzoic acid ring system and aroyl hydrazones, novel set veratric acid derivatives were designed as potential antimicrobial agents by using molecular hybridization approach for further synthesis and biological evaluation.

Chemistry: Materials and Method

The 1H NMR spectra were recorded on 'Joel MYFT' 60 MHz using Tetra methyl silane (TMS) as internal standard whereas IR spectrum was recorded on FTIR ('Perkin Elmer Spectrum RX1') by preparing a pellet using KBr ('KBr method'). Melting points for the some of the synthesized starting materials and final compounds are recorded on a 'Thermonic' melting point apparatus. All starting materials and reagents for the synthesis were purchased from

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commercial sources and all the reactions as well as chromatographic processes were monitored by TLC technique on pre-coated silica gel plates of size 3cm x 1cm procured from E-Merck., followed by exposure to iodine vapors and/ or UV irradiation for detection and/or anisaldehyde reagent then it is charred or heated on a hot plate. Distilled solvents were used for carrying out reactions as well as column chromatography.

Synthesis

Step 1

Procedure for synthesis of Ethyl veratrate: A mixture of veratric acid (0.08 mol) and ethanol (0.74 mol) was heated under reflux in the presence of sulphuric acid (0.2 ml) in dry benzene (100 ml) using a Dean–Stark water separator. Excess benzene was evaporated in vacuo. The resulting residue was triturated with saturated NaHCO3 solution until CO2 evolution ceased. The crude product obtained was finally recrystallized.

Molecular formula- C11H14O4, Yield (%) - 78 , M.P- 34-36 oC, Rf.-0.43, IR (KBr) 3082, 1711, 1513, 1024 1H NMR7.688-6.845(m, 3H), 4.451-4.096 (q, 2H),1.413-1.177 (t, 3H), 3.828 (s, 6H)

Step 2

Procedure for synthesis of Veratric acid Hydrazide synthesis: A solution of ethyl veratrate (10 mmol) in triethylamine and ethanol was heated under reflux with hydrazine hydrate (30 mmol) for 4-5 h. The reaction mixture was allowed to attain room temperature and the deposited solid was filtered, washed, dried, and recrystallized.

Molecular formula- C9H12 N2O3, Yield %- 74, M.P.- 143-145 0C, Rf.-0.64, IR (KBr) 3435, 2967, 1697, 1275, 1H NMR7.407-6.780 (m, 3H), 3.905 (s, 6H)

Step 3

General procedure for synthesis of veratric acid derivatives: An equimolar amount of appropriate aromatic aldehyde was added to a solution of hydrazide derivatives in 20 mL of ethanol, in the presence of catalytic amount of hydrochloric acid. The reaction was stirred for 2-3 hours at reflux, and the solvent was evaporated under reduced pressure. The colored precipitate was collected by filtration, washed with cold water and dried under vacuum to give the desired N-acylhydrazone derivatives that were purified by recrystallization in ethanol/DMF yielding compounds in excellent yields.

It was observed that:

• The rate at which these imine-like compounds are formed is generally greatest near a pH of 5, and drops at higher and lower pH's.

• These derivatives are easily prepared and are often crystalline solids - even when the parent aldehyde or ketone is a liquid.

Biological Evaluation

All the synthesized veratric acid derivatives were screened for their antimicrobial activity against bacterial strains. The resazurin assay utilising microtitre-plate used for determination of minimum inhibitory concentration (MIC) values of synthesized compounds against bacterial strains.

General: Incubator at 35 to 37 °C; pipettes of various sizes; sterile tips, 100, 200, 500, and 1000 µL; 5 mL multichannel pipette; centrifuge tubes; vortex mixer; centrifuge; petridishes; sterile universal bottles; UV spectrophotometer (Shimadzu); sterile resazurin powder; sterile normal saline; sterile nutrient broth (I. P.) (Hi-media); antibiotic solutions (Sigma– Aldrich); sterile solution of 10% (v/v) DMSO in water (Sigma–Aldrich).

Medium: Nutrient broth medium (I. P.) used for susceptibility testing of bacterial strains.

Use of Standardised Bacterial Colony Numbers: To ensure that a uniform number of bacteria were always used, a set of graphs of killing/viability curves for each strain of bacterial species was prepared. A final concentration of 5 × 105 cfu/mL was adopted for this assay. Thus different strains and different bacterial species could be compared.

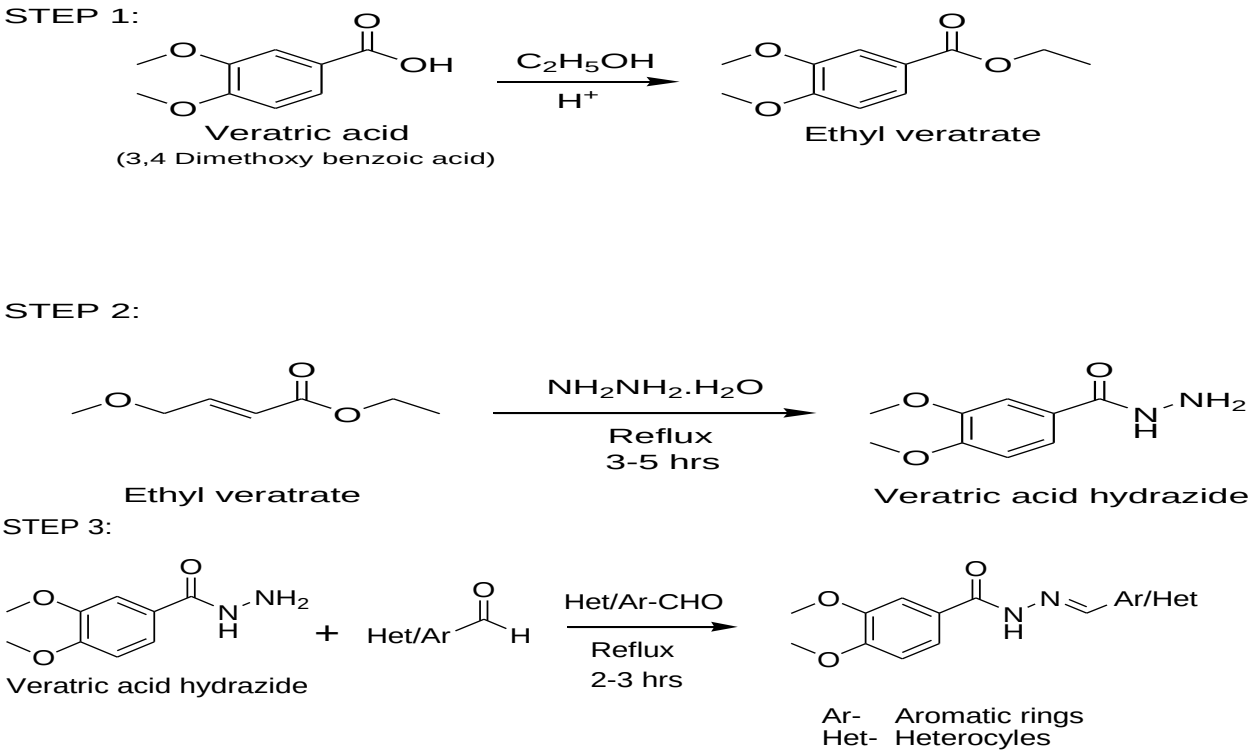
Preparation of Bacterial Culture: Using aseptic techniques a single colony was transferred into a 20 mL bottle of nutrient broth, capped and placed in incubator overnight at 37 °C. The optical density was recorded at 500 nm, and serial dilutions were carried out with appropriate aseptic techniques until the optical density was in the range of 0.5–1.0. The actual number of colony forming units was calculated from the viability graph. The dilution factor needed was calculated and the dilution was carried out to obtain a concentration of 5 × 106 cfu/mL.

Preparation of Resazurin Solution: The resazurin solution was prepared by dissolving a 3 mg in 50 mL of sterile distilled water.

Preparation of The Plates: Plates were prepared under aseptic conditions. A sterile 96 well plate was labeled. A volume of 100 µL of test material in DMSO or sterile water (usually a stock concentration of ~5 mg/mL for test compounds) was pipetted into the first row of the plate. To all other wells 100 µL of nutrient broth was added. Serial dilutions were performed using a multichannel pipette. Tips were discarded after use such that each well had 100 µL of the test material in serially descending concentrations. Using a pipette 30 µL of 3.3 × strength broths was added to each well to ensure that the final volume was single strength of the nutrient broth. 10 µL of bacterial suspension (5 × 106 cfu/ mL) was added to each well to achieve a concentration of 5 × 105 cfu/mL. Plate was kept for 24 hours depending upon organism. To each well 30 µL of resazurin indicator solution was added. Each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. Each plate had a set of controls: a column with a broad-spectrum antibiotic as positive control (ciprofloxacin in serial dilution), a column with all solutions with the exception of the test compound, and a column with all solutions with the exception of the bacterial solution adding 10 µL of nutrient broth instead. The plates were prepared in duplicate, and placed in an incubator set at 37 °C for 24 h. The colour change was then assessed visually. Any colour changes from purple to pink or colourless were recorded as positive. The lowest concentration at which colour change occurred was taken as the MIC value. The average of three values was calculated and that was the MIC for the test material and bacterial strains.

Result and disscussion

Novel set of molecules were designed by rational design strategy of new ligands or prototypes based on the recognition of pharmacophoric sub-unities in the molecular structure of two or more known bioactive derivatives which, through the adequate fusion of these sub-unities, lead to the design of new hybrid architectures that maintain pre-selected characteristics of the original templates. The technique is known as molecular hybridization technique. Here two fused pharmacophore were veratric acid moiety and aroyl hydrazone. The designed molecules were successfully synthesized in the laboratory using literature methods and structures were confirmed by NMR and IR spectroscopy.

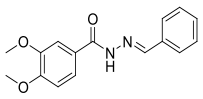
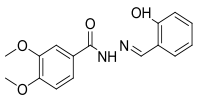
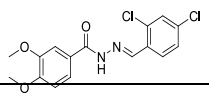
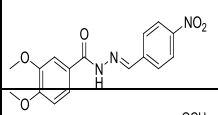
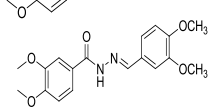
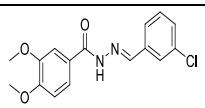


VNT_VH-1 - Ar = Phenyl
VNT_VH-2 - Ar = 2-hydroxy phenyl
VNT_VH-3 - Ar = 2,4-dichloro phenyl
VNT_VH-4 - Ar = 4-nitro phenyl
VNT_VH-5 - Ar = 3,4-dimethoxy phenyl
VNT_VH-6 - Ar = 3-chloro phenyl
VNT_VH-7 - Ar = 4-hydroxy phenyl
VNT_VH-8 - Ar = 4-methoxy phenyl
VNT_VH-9 - Ar = 2,5-dimethoxy phenyl
VNT_VH-10 - Ar = 4-Isopropyl phenyl
VNT_VH-11 - Ar = 4-Fluoro phenyl
VNT_VH-12 - Ar = 3-hydroxy 4-methoxy phenyl
VNT_VH-13 - Het = 2- furyl
VNT_VH-14 - Het = 2- Pyrrole
VNT_VH-15 - Het = 2- thienyl
VNT_VH-16 - Het = 2- pipernyl
VNT_VH-17 - Ar = cinnamyl
VNT_VH-18 - Het = 2- Pyridinyl

Scheme 1: Synthesis Of Veratric Derivatives

Characterization

Table 1. Physico-chemical properties of synthesized test compounds

	Compound	Molecular Formula	Molecular Weight	M.P(^{0}C)	Yield (%)	Spectral data
1.		$C_{16}H_{16}N_2O_3$	284	165	84	IR- 3214, 2941, 1635, 1512 ^{1}H NMR - 11.069 (s, 1H), 8.330(s, 1H), 7.546-780(m, 3H), 7.729-7.439(m, 5H), 3.970(S, 6H)
2.		$C_{16}H_{16}N_2O_4$	300	142	82	IR- 3217, 3082, 2926, 1646 ^{1}H NMR - 10.216 (s, 1H), 8.413 (s, 1H), 7.388-7.242(m, 2H), 6.970-6.928 (m, 2H), 3.885(S, 6H), 3.551(s, 1H)
3.		$C_{16}H_{16}Cl_2N_2O_3$	253	187	90	IR - 3197, 2824, 1645 ^{1}H NMR – 9.398 (s, 1H), 8.575 (s, 1H), 7.557-6.987 (m, 6H), 3.954(s, 6H),
4.		$C_{16}H_{16}N_3O_5$	329	212	78	IR-3156, 2928, 1649 ^{1}H NMR – 11.312 (s, 1H), 8.502(s, 1H), 8.347-7.867(m, 4H), 7.578-690(m, 3H), 3.974(s, 6H)
5.		$C_{18}H_{20}N_2O_5$	344	175	83	IR--3196, 2947, 1636 ^{1}HNMR – 9.480 (s, 1H), 8.437 (s, 1H), 7.669-(s, 1H), 7.324-6.887 (m, 3H), 3.925-3.915 (s, 12H)
6.		$C_{16}H_{15}N_2O_3$	316	147	77	IR-3196, 2927, 1646 ^{1}HNMR – 10.567 (s, 1H), 8.289 (s, 1H), 7.994(s, 1H), 7.712-7.460(m, 3H), 7.619- 7.170(m, 3H), 3.926(s, 6H)

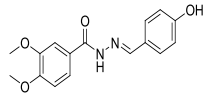
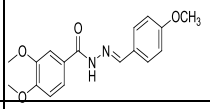
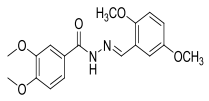
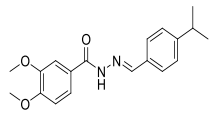
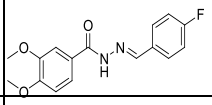
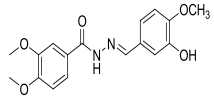
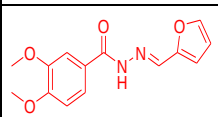
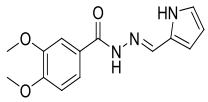
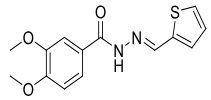
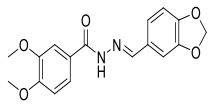
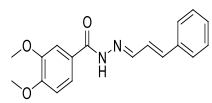
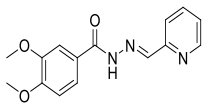
7.		$C_{16}H_{16}N_2O_4$	300	234	92	IR--3261, 2949, 1644 ^{1}HNMR – 11.174 (s, 1H), 8.418 (s, 1H), 7.782-6.857 (m, 4H), 7.578-6.808(m, 3H), 3.934(s, 6H)
8.		$C_{17}H_{18}N_2O_4$	314	184	84	IR-3256, 2925, 1642 ^{1}HNMR – 11.220 (s, 1H), 8.782 (s, 1H), 7.557-6.894 (m, 3H), 7.753-7.008 (m, 4H), 3.942 (s, 6H), 3.840 (s, 3H)
9.		$C_{18}H_{20}N_2O_5$	344	178	79	IR-3268, 2925, 1647 ^{1}H NMR – 11.002(s, 1H), 8.612(s, 1H), 7.559-6.821(m, 2H), 7.432-6.921(m, 3H),3.960 (s, 6H),
10.		C19H22N2O3	326	153	87	IR – 3260, 2938, 1643 1HNMR - 9.483 (s, 1H), 8.217 (s, 1H), 7.716-7.292 (m, 4H), 7.577-6.808 (m, 3H), 3.925 (s, 6H), 1.307-1.193 (d, 6H), 3.094-2.716 (m, 1H)
11.		C16H15FN2O3	302	190	73	IR-3256, 2928, 1645 1HNMR – 11.194 (s, 1H), 8.392(s, 1H), 7.895-7.232 (m, 4H) , 7.557-6.865(m, 3H), 3.946 (s, 6H)
12		C17H18N2O5	330	194	80	IR-3255, 2923, 1648 1HNMR – 10.988(s, 1H), 8.433(s, 1H), 7.547-6.845 (m, 3H) , 7.358-6.624 (m, 3H), 3.997 (s, 1H), 3.862 (s, 3H), 3.514 (s, 1H)
13		C14H14N2O4	274	202	87	IR-3259, 1567, 1645 1HNMR – 12.119 (s, 1H), 11.001 (s, 1H), 8.338(s, H), 7.729-7.439(m, 6H), 7.021 (s, 1H)
14		C14H15N3O3	275	195	73	IR--3255,1536, 1649 1HNMR – 11.839 (s, 1H), 11.394 (s, 1H), 8.442(s, 1H), 7.953 (s, 1H) , 7.870-7.739(m, 2H), 6.874 (s, 1H)
15		C14H14N2O3S	290	208	79	IR-3260, 2923, 1539, 1640 1HNMR – 11.931 (s, 1H), 11.490 (s, 1H), 8.437 (s, 1H), 7.469(s, 1H), 7.388-7.242(m, 2H), 6.970-6.928 (m, 2H), 2.854 (s, 1H
16		C17H16N2O5	328	190	86	IR-3255, 2923, 1532, 1648 1HNMR - 12.281 (s, 1H), 11. 677 (s, 1H), 8.735(s, 1H), 8.389 (s, 1H), 7.442-7.012 (m, 3H), 3.816 (s, 3H), 3.753 (s, 3H)
17		C18H18N2O3	310	225	92	IR – 3264, 2928, 1658 1H NMR- 11.132 (s, 1H), 7.541-7.39 (m, 3H), 6.975-6.702 (dd, 2H)
18		C15H15N3O3	285	170	87	IR- 3217,2978, 1642, 1517 1H NMR- 11.384 (s, 1H), 8.193 (s, 1H), 7.545-6.824 (m, 3H), 6.434-6.226 (m, 4H)

Table 2. Antibacterial test results: (MIC µg/ml)

Compound VNT_VH	E. Coli.	S. Aureus
1	56.25	112.5
2	26.25	52.5
3	37.5	75
4	33.13	28.25
5	58.5	48.125
6	62.5	>125
7	41.25	62.5
8	20.625	41.25
9	52.5	105
10	68.75	>125
11	32.5	26.5
12	21.25	42.5
13	65	>125
14	21.25	42.5
15	50	100
16	41.25	82.5
17	48.13	68.75
18	76.25	105

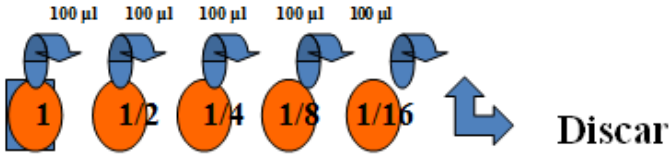


Figure 1. 8 Serial dilution

Conclusion

The molecules designed were synthesized in the laboratory using methods illustrated in the literature and structures were then confirmed by NMR and IR spectroscopy. All the synthesized compounds were screened for their antimicrobial activity against bacterial strains. Some of the Compounds VNT_VH 2, 3, 4, 11 showed good inhibition against S. aureus and E. coli species, especially VNT_VH 8, 12, 13 showed activity nearly equivalent to that of standard drug streptomycin against E.coli.

Disclosure

The authors report no conflicts of interest in this work.

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