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One pot facile synthesis of flavanoidal oxadiazinanones: In vitro antibacterial activity, docking and MD simulation using DNA gyrase

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Abstract:

Flavanones are commonly spread in nature because they are essential intermediates in the biosynthetic flavonoid pathway1 and are of therapeutic significance because of their broad variety of biological activities such as hypertensive, antifungal, antibacterial, and antitumor activities2. Therefore, the field of flavanones is of numerous attentions to medicinal chemists for drug discovery. Flavanones have a 2,3- dihydro skeleton in the C6-C3-C6 structure of the flavonoid and do not have a double bond between C2 and C3, (Fig. 1) which renders them chiral in the C2 position3 hence two stereoisomeric forms of each flavanone are possible4. The chirality means that the B-ring is distorted compared to the A-C rings and is not planar. Such a disparity in molecular orientation is of considerable importance as it may influence how flavonoids engage with biological receptors and their bioactive properties5,6.

Keywords: Flavanones, structure, stereoisomeric, bioactive properties, biological

Introduction

The literature of the molecules possessing oxadiazinanones moiety reveals their biological importance and hence several reports on the synthesis of oxadiazinanones derivatives with varying yields are available7,8. Oxadiazinanone ring has boat conformation9 and the nitrogen present in the ring shows the conformational mobility10, which is responsible for their bioactivity. Such compounds are excellent candidates for the production of biologically active molecules that can serve as a lead component in the creation of anticancer, antimicrobial, and antioxidant drugs due to the presence of flexibility or molecular adaptability in the oxadizinanone ring11,12. Certain enzymes inside the microbial cell are the target for most of the antimicrobial agents to prevent their activity. DNA gyrase is the well-known and authenticated target for the establishment of new antimicrobial agents and its binding involvement with the lead molecules serves as a key role in the development and discovery of drugs13–15. Molecular docking studies16, MD simulation17 and scanning electron microscope18 play an essential role in the development and discovery of drugs. With the application of previous studies19, we have planned to present an efficient synthesis of flavanoidal oxadiazinanone such as 2-phenylspiro[chroman-4,2'-[1,3,4]oxadiazinan]-5'-one (6),

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2-(2-hydroxyphenyl)spiro[chroman-4,2'-[1,3,4]oxadiazinan]-5'-one (7), 2-(3-hydroxyphenyl)spiro[chroman-4,2'-[1,3,4]oxadiazinan]-5'-one (8), 2-(4-hydroxyphenyl)spiro[chroman-4,2'-[1,3,4]oxadiazinan]-5'-one (9), 7-hydroxy-2-phenylspiro[chroman-4,2'-[1,3,4]oxadiazinan]-5'-one (10) as potential anti-bacterial agent. Adding a feather to our awareness, about the synthesis of flavanoidal oxadiazinanone (6-10) the reaction of flavanone and its derivatives (1-5) with cyanoacetohydrazide has been portrayed along with the molecular docking, MD simulation and scanning electron microscopy were also performed.

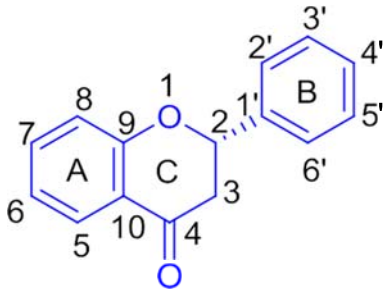

Fig. 1: Structure of flavanone with appropriate numbering of atoms and rings

Experimental section

Materials and methods

Chemicals were purchased from Sigma-Aldrich Chemicals Pvt. Ltd. and purity was confirmed by thin-layer chromatography. Melting points recorded on Kofler apparatus in degree celsius. ^{1}H NMR spectra and ^{13}C NMR spectra were run in DMSO-d6 on a BRUKER Avance NEO 500 MHz NMR spectrometer with the standard. Chemical shift values reported in ppm (δ) concerning solvent peak and J values reported in Hertz. The FT-IR spectra were recorded on KBr pellets with PerkinElmer FT-IR spectrometer spectrum (Version 10.4.00) instrument and values obtained in cm^{-1}. Thin Layer Chromatography (TLC) plates coated with Silica Gel G were used to monitor the homogeneity and success of the reaction while exposed to iodine vapour.

General methods for the synthesis of flavanoidal oxadiazinanone derivatives (6-10)

Cyanoacetohydrazide was added in the same solvent to a 1 mmol solution of flavanones such as flavanone (1), 2'- hydroxyflavanone (2), 3'- hydroxyflavanone (3),
4'- hydroxyflavanone (4), 7- hydroxyflavanone (5) in acetic acid (30 mL) in the equimolar ratio.

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The reaction mixture was stirred on a magnetic stirrer, and a white precipitate was formed after 3 hours. After completion of the reaction, the precipitate was suspended in water (30 mL), neutralized with saturated aqueous sodium bicarbonate (NaHCO3), and filtered using suction. Anhydrous sodium sulphate is used as a drying agent. The compounds 2-phenylspiro[chroman-4,2'-[1,3,4] oxadiazinan]-

5'-one (6), 2-(2-hydroxyphenyl)spiro[chroman-4,2'-[1,3,4]oxadiazinan]-5'-one (7), 2-(3-hydroxyphenyl)spiro[chroman-4,2'-[1,3,4]oxadiazinan]-5'-one (8), 2-(4-hydroxyphenyl)spiro[chroman-4,2'-[1,3,4]oxadiazinan]-5'-one (9), 7-hydroxy-2-phenylspiro[chroman-4,2'-[1,3,4]oxadiazinan]-5'-one (10) were obtained in powder form by removing the solvent. The above desired compounds (6-10) obtained as precipitate/ solid compound were purified by recrystallization from ethanol. Silica Gel G coated TLC plates were used to confirm the purity and success of the reaction. TLC plates showed a single spot when exposed to iodine vapour in the iodine chamber.

Results and discussion

Chemistry

To develop new flavonoidal heterocycles that may be of use in the design of modern, effective, selective and less toxic antibacterial agents, we herein present the synthesis of flavonoidal oxadiazinanones (6-10) from flavanones (1-5) with a versatile and active reagent cyanoacetohydrazide (Scheme 2.1). Cyanoacetohydrazide can serve as an ambident nucleophile i.e., both N- and C- nucleophilic.

For the synthesis of numerous heterocyclic compounds of different ring sizes having nitrogen atom, cyanoacetohydrazide and its analogues are essential starting materials or intermediates. The presence of five active positions contributes to a variety of polyfunctional heterocyclic compounds with distinct reactants36. The reaction does not involve a thorough drying of solvents, reagents or inert atmosphere. The structural elucidation of the newly synthesized compounds (6-10) was done based on FT-IR, 1H NMR, 13C NMR and LC-MS spectra. In the FT-IR spectra of compounds (6-10), absorption bands in the region between 3329-3371 cm-1 and due to OH stretching, whereas NH stretching in the region between 3184-3259 cm-1 for all compounds.

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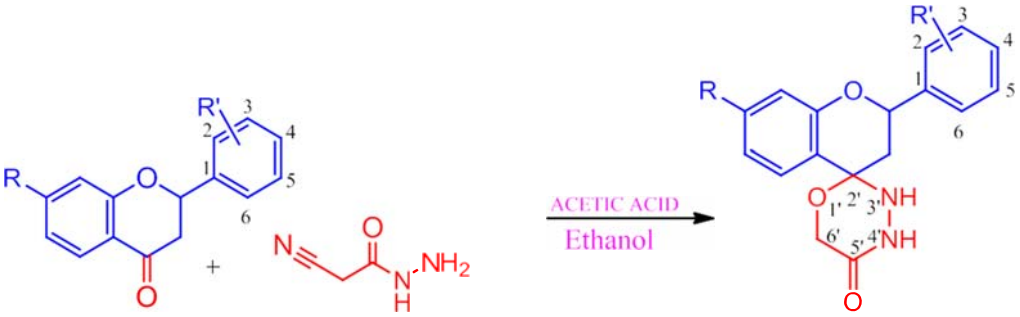

R	R'	R	R'	
H	H	(6)	H	Hs
H	2-OH	(7)	H	2-OH
H	3-OH	(8)	H	3-OH
H	4-OH	(9)	H	4-OH
OH	H	(10)	OH	H

Scheme 2.1: Synthesis of flavanoidal oxadiazinanones (6-10)

The strong absorption band in the region between 1673-1704 cm-1 and a weak absorption band in the region between 1604-1626 cm-1 confirms the presence of C=O and C=C, respectively. The shifting of C=O absorption band in the FT-IR spectra of all synthesized compounds and presence of weak band in the region between 1456- 1460 cm-1 confirms the formation of oxadiazinanone. During SN2 cyclization process C≡N (soft leaving group) leaves to generate the six member heterocyclic product because carbon bonded to the C≡N attracts the pair of electrons on hydroxyl group which confirms by the non-appearance of absorption band at 2200-2250 cm-1 in the FT-IR spectra of all synthesized compounds. Furthermore, a strong band at 1121- 1124 cm-1 ascribed the ether linkage formed during product formation. Since the formation of synthesized compounds (6-10) may be understood by the nucleophilic attack of hydroxyl group of flavanone produced by the attack of rear nitrogen of amino group of cyanoacetohydrazide (Scheme 2.2). In the 1H NMR spectra of the compounds (6-10) two singlets (exchangeable with D2O) for two protons of 2 × NH observed at δ 2.50-2.51 and 7.82-8.01 ppm. Two distorted doublets at δ 3.80-3.85 and 4.22-4.32 ppm ascribed to the two protons (Ha, Hb) of the methylene group (OCH2) between ether linkage and ketonic group of oxadiazinanone ring in synthesized compounds. Signals of three one-proton double doublets for H-2, Hax-3 and Heq-3 typical of a flavanone at δ 5.12-5.35 (1H, dd, J = 14.5, 3.0 Hz, H-2), 3.18-3.42 (1H, dd, J = 20.0, 3.0 Hz, Heq-3) and 2.64-2.82 (1H, dd, J = 20.0, 14.0 Hz, Hax-3). In the

case of (6-10) independent singlets of phenolic OH resonates in the range of δ 9.55-

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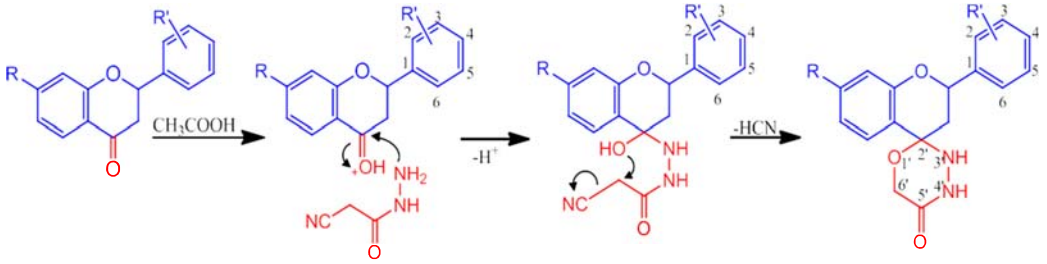
10.03. Aromatic protons of rings A and B show the multiplets in the range of δ 6.36-7.56. 13C NMR spectra also show the good agreement with the formation of compounds (6-10) and their signals support the structures proposed for synthesized compounds. All synthesized compounds displayed signals at δ 165.1-165.6 confirm the presence of C=O group. Signals at δ 76.0-76.6 due to methylene group (CH2) of oxadiazinanone ring were also present. LC-MS also support the structure of synthesized compounds (6-10). In the mass spectra of (6), (7), (8), (9) and (10)

molecular ion peak (M+.) appeared at m/z 296, 312, 312, 312, 312 respectively.

Scheme 2.2: Conceivable reaction mechanism for the construction of desired products (6-10).



Scanning electron microscopy

Scanning electron microscopy (SEM) is the approach where only milligrams of substance can be utilized to assess particle size, shape, and texture. The SEM signals include details on the exterior morphology (texture), crystalline structure, homogeneity, thickness and orientation of the nano compound materials.SEM analyzed the surface morphology of nano oxadiazinanones (6-10). The images of nano oxadiazinanones (6-10) have width of 14 mm and a magnification of 3.00 KX for (6-8), 15.00KX for (9) and 5.00 KX for (10) (Fig. 2). SEM images predicted that the homogeneous crystals of nano oxadiazinanones (6-10) were approximately needle-shaped. The particles agglomerate was formed by the edge-to-edge conjoining with a few microcrystals.

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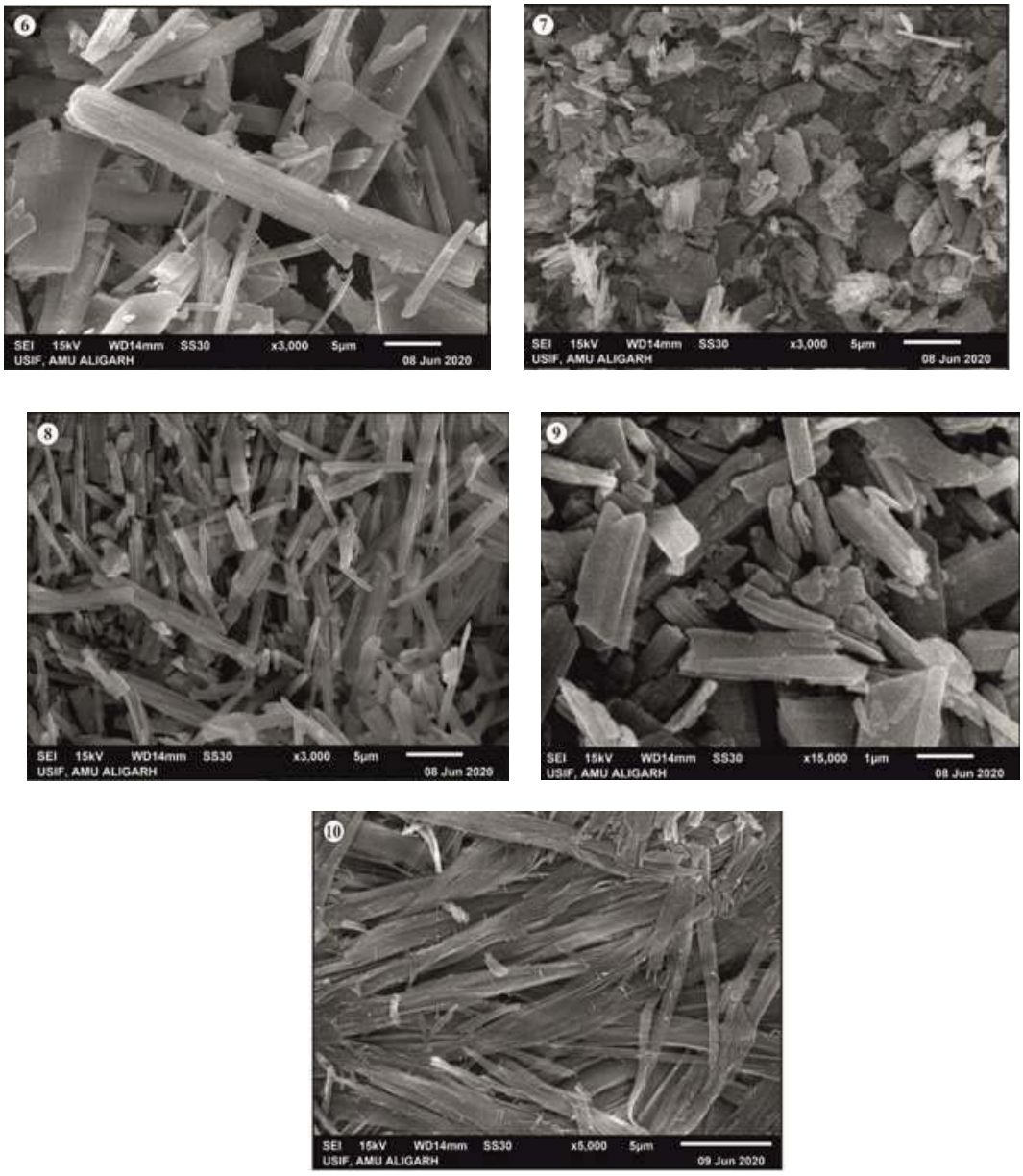

Fig. 2: SEM images showing surface morphology and crystalline nature of synthesized compounds (6-10).

Drug-likeness and bioactivity score

The conception of drug-like and lead-like chemical properties has a substantial impact on the development of compounds for high-throughput screening and on the creation of lead libraries37. 'Lipinski's rule of five' proposed by the medicinal chemist Christopher A. Lipinski and his colleagues are a rule of thumb to search drug-like molecules based

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on their molecular properties38. The rule proposes that the compound is highly probable to be membrane-permeable and readily absorbed by the body if its molecular weight (Mw) is less than 500, the lipophilicity of the compound, represented as log P is less than 5, the number of groups capable of contributing hydrogen atoms to form hydrogen bonds (HBD) is less than 5, the number of groups capable of receiving hydrogen atoms to form hydrogen bonds (HBA), is less than 1039. Table 1 includes the Lipinski parameters estimated for the synthesized oxadiazinanone derivatives (6-10). Hydrogen-bonding capacity is an important factor in describing drug permeability40. When the number of H-bond donors and H-bond acceptors are more than 5 and 10 respectively, poor permeation or absorption more likely to be observed. In the all synthesized compounds (6-10) number of hydrogen bond donors are 2 to 3 (<5), hydrogen bond acceptors are 5 to 6 (<10), the value of log P ranges from 1.88 to 2.39 (<5) and value of molecular weight ranges from 296.33 to 312.32 (<500). Based on these results, all the synthesized compounds (6- 10) follow the five-requirements of Lipinski rule; therefore, it can be concluded that all compounds will be readily absorbed and have good permeability.

Table 1: Calculated physicochemical properties of synthesized flavanoidal oxadiazinanone derivatives (6-10)

Compound	Mwa	MilogPb	TPSAc	HBDd	HBAe	No. of violations
6	296.33	2.39	59.59	2	5	0
7	312.32	2.33	79.82	3	6	0
8	312.32	1.88	79.82	3	6	0
9	312.32	1.91	79.82	3	6	0
10	312.33	1.88	79.82	3	6	0

aMolecular weight, bLogarithm of partition coefficient, cTopological surface area, dHydrogen bond donor, eHydrogen bond acceptor

The probability of the investigating compound to be active, moderately active or inactive may be predicted by bioactivity score. The bioactivity of synthesized compounds based on: (a) bioactivity score > 0.00, corresponds to active, (b) bioactivity score > -0.50, corresponds to moderately active, (c) bioactivity score < - 0.50, corresponds to inactive. Table 2 shows the results of synthesized compounds (6-10), which predicted that some compounds are moderately active and some are inactive.

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Table 2: Bioactivity score of synthesized flavanoidal oxadiazinanone derivatives (6- 10)

Compound	GPCR Ligand	Ion Channel	Kinase Inhibitor	Nuclear Receptor Ligand	Protease Inhibitor	Enzyme Inhibitor
6	-0.28	-0.65	-0.52	-0.27	-0.28	-0.30
7	-0.23	-0.59	-0.47	-0.16	-0.23	-0.23
8	-0.21	-0.57	-0.45	-0.10	-0.23	0.23
9	-0.20	-0.56	-0.43	-0.11	-0.21	-0.23
10	-0.21	-0.58	-0.42	-0.09	-0.23	-0.23

Conclusion

Oxadiazinanone derivatives have been synthesized using readily available starting materials flavanone derivatives and cyanoacetohydrazide employing a one-pot methodology. In vitro antimicrobial and in silico docking studies of all synthesized compounds have been performed. The results reveal that all synthesized compounds show effective antibacterial activity against various Gram-negative bacterial strains. Studies of molecular docking explained how synthesized compounds interact with DNA gyrase. In addition, MD simulation technique demonstrated the binding stability of synthesized compounds against DNA gyrase and showed promising results.

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