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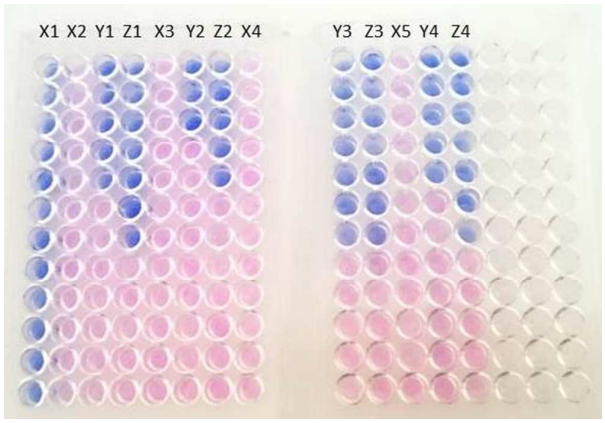

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ARTICLE TYPE

A bluish – green emitting organic compound Methyl 3–[(E)–(2–hydroxy–1-naphthyl)methylidene]carbazate: Spectroscopic, thermal, fluorescence, antimicrobial and molecular docking studies

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The present paper describes the physicochemical properties and biological activities of an organic single crystal, Methyl 3–[(E)–(2–hydroxy–1-naphthyl)methylidene]carbazate which was grown by slow evaporation solution growth technique. Powder X-ray diffraction study of the compound was carried out and the (hkl) values are found to be comparable with the reported value. Vibrational properties of the compound were analysed on the basis of FT-IR and FT-RAMAN spectra. Proton NMR spectrum was consistent with the chemical structure of the compound. From UV-VIS-DRS analysis and fluorescence studies, the absorption range and the bluish green emission property of the title compound were found. The melting point of the compound was found to be at 205° C from the TG – DTA – DSC analysis. The antimicrobial activities of the title compound was screened using resazurin reduction assay against human pathogenic bacteria such as *Shigella dysenteriae*, *Vibrio Cholerae*, *Streptococcus faecalis*, *Bacillus cereus* and fungi such as *Candida krusei*, *Candida albicans*, *Candida glabrata*. The molecular docking demonstrated that the title compound could bind well with the active site of human estrogen receptor and act as a potential inhibitor of ERα and ERβ.

Introduction

Nonlinear optical single crystals have their applications in high – energy laser for inertial confinement fusion research, color displays, electro – optical switches, frequency generations, etc.[1] Organic second order nonlinear optical chromophores and polymers have been the subject of extensive research for their range of applications optical communication, optical interconnects for computing and for the generation of THz radiation.[2] Fluorescent molecules have played a key role in chemical, biological and medical sciences.[3] Fluorescence in aldehydes and ketones arise from the occurrence of low lying n- π* transition associated with the carbonyl group which favours high yields of singlet to triplet intersystem crossing. Naphthaldehyde has analytical interest because of the fluorogenic properties of the naphthyl group and the high reactivity of the carbonyl group which is useful in the fluorescent derivatives of the non- fluorescent analytes.[4] The naphthalene group as a fluorophore has been studied extensively due to its characteristic photophysical properties. The Schiff base compounds can be classified according to their photo and thermochromic characteristics. 2- hydroxyl Schiff base ligands are of interest due to the resistance (O – H....N & N – H ....O) type hydrogen bonds and tautomerism between the enol – imine and keto – enamine forms.[3] A vast array of Schiff bases having excellent activity in broad spectral range forms an invaluable part of the present anti-microbial, antifungal, antitubercular, anticancer and anti – HIV of the clinicians. The Schiff bases exhibit a variety of biological therapeutic properties and fastest growing antibacterial class in terms of global revenue, increasingly being used in both the hospital and community sectors to test the broad range of infections.[5]

Breast Cancer, is the second most wide spread type of cancer after the lung cancer with the rate of 10.4% and also in 2008, 458,503 deaths caused due to breast cancer (World health organization international agency for research on cancer). Breast cancer is 100 times more common in men.[6,7] Both normal and breast cancer cells have receptors to bind estrogen and progesterone circulating in the blood.[8]

Estrogen interacts with the estrogen receptors ERα and ERβ, and it controls multi functions in mammalian tissues. It also plays an important role in female reproduction, bone formation, and cardio vascular and Central Nervous System (CNS) health.[9] Selective Estrogen Receptor Modulators (SERMs) on Schiff base compounds act as an alternative approach for Hormone Replacement Therapy (HRT).[10] The breast cancer cells are estrogen receptor positive and more likely to respond hormonal therapies with Tamoxifen, Raloxifene, and Toremifene. These cells also have a better prognosis than cancers that are hormone receptor negative.[8] Tamoxifen (Nolvadex R) is a drug, taken orally as a tablet, which interferes with the activity of estrogen. The serious side effects of Tamoxifen include blood clots, stroke,

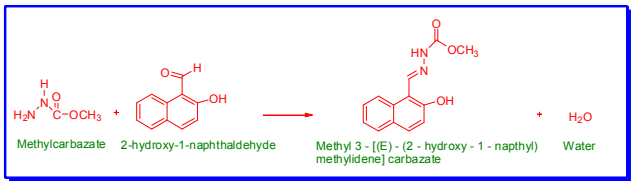
uterine cancer, and cataracts. Side effects of Raloxifene are serious blood clots in the legs, lungs, or eyes. Other reactions including leg swelling/pain, trouble breathing, chest pain, vision changes. Therefore these side effects make these drugs resistance for use and require studies on a better alternate.

Methyl 3–[E]–(2–hydroxy–1- napthyl) methylidene] carbazate (MNMC) is such a Schiff base compound with the molecular formula $C_{13}H_{12}N_2O_3$. Sheng et.al[11] have already reported the structural studies of this compound. The molecule, adopts an *E* or *trans* configuration with respect to the C=N bond. The crystal structure of MNMC is stabilized by intramolecular O—H···N interaction and intermolecular N—H···O interaction.[11] In the present investigation, elaborate studies on the spectral, thermal, electrochemical and fluorescent properties of the title compound have been made. The antimicrobial activity of the Schiff base ligand was screened against selected kinds of bacteria and fungi and the minimum inhibitory concentration (MIC) against the human pathogenic bacteria such as Shigella dysenteriae, were analysed by the resazurin reduction assay described by Sarker et al.[12] The molecular docking of MNMC Schiff base was carried out with human estrogen receptor as target protein.

EXPERIMENTAL

Materials and Methods

The title compound was synthesized by following the procedure given in literature by Sheng et.al.[11] A solution of 1 mmol of methyl carbazate (Sigma aldrich) in 5 ml of ethanol was added slowly to a solution of 1 mmol of 2-hydroxy-1-naphthaldehyde (sigma aldrich) in 15 ml absolute ethanol, under heating and stirring. The mixture was refluxed for 3 h, then cooled to room temperature. Yellow block-shaped crystals were formed on slow evaporation of the solvent. The reaction scheme is shown in Scheme 1.



Scheme 1 Reaction scheme for MNMC

The obtained Methyl 3–[(E)–(2–hydroxy–1-napthyl) methylidene] carbazate (MNMC) crystals were further purified by recrystallization process using ethanol and dimethylformamide as solvents. Yellow block shaped MNMC single crystals (Fig. 1a & 1b) were obtained after a period of 3 months. The morphology of MNMC single crystal (Fig. 1c) was predicted using WinXMorph.[13, 14] The shape of MNMC single crystal was analysed using Apex Bruker Software (Fig. 1d). All the characterization studies were performed on the MNMC single crystal grown from ethanol solvent.

Characterizations

Single crystal X – ray diffraction analysis was carried out on the grown MNMC singe crystal using Enraf (Bruker) Nonius CAD4 Single Crystal X - ray Diffractometer. D2 PHASER Powder X-ray diffractometer is used to analyse the cell parameter and the (hkl) planes. Alpha Bruker FT – IR in the region 400 – 4000 cm^{-1} and Bruker 27 multiRAM stand alone FT – RAMAN spectrometer with scanning range 50 – 4000 cm^{-1} were used for analysing the spectral properties of the compound. The proton NMR study of MNMC compound was performed using Bruker AVANCE III 500 MHz multinuclei solution spectrometer.Shimadzu 2450 UV-VIS Spectrophotometer was utilized for the study of the electronic absorption range of the compound in the region 200 – 800 nm. Cyclic voltammetry measurement was made for the title compound using CHI 600D electrochemical analyzer (room temperature) with 3 electrode cell in a solution of $BuNClO_4$ in dichloromethane at a scanning rate of 100 mVS^{-1}.Glassy carbon electrode was used as working electrode, platinum foil was used as counter electrode and reference electrode Ag / AgCl was calibrated after each measurement using ferrocene (Fc). The fluorescence study was carried out using Jobin Yvon fluorolog – 3 – 11 spectroflurimeter having Xenon lamp with 450 W as source in the range 180 – 1550 nm with resolution of 0.2 nm.

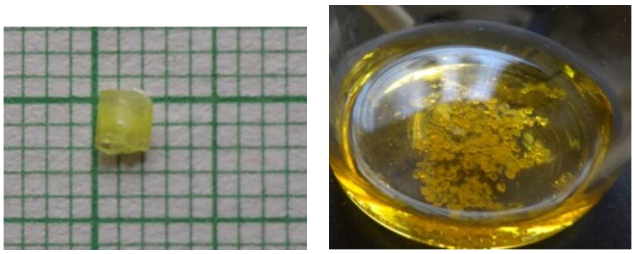


Fig. 1 As grown single crystals of MNMC (a) from ethanol solvent

(b) from dimethyl formamide solvent

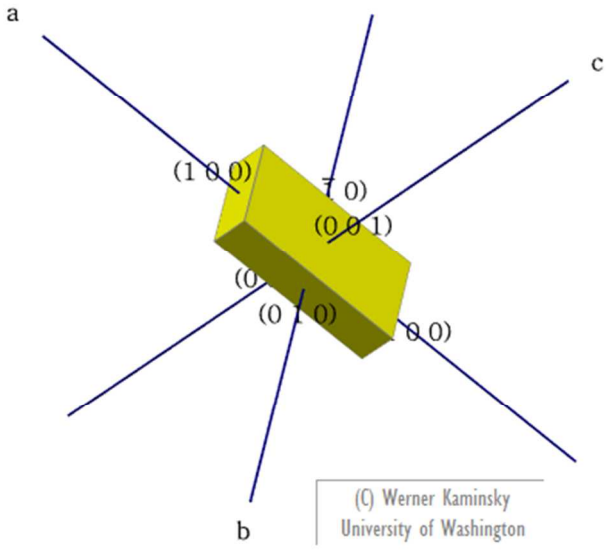


Fig. 1c Morphology of MNMC single crystal

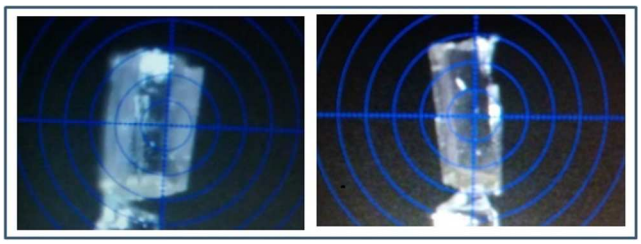


Fig. 1d Shape of MNMC single crystal in different orientations

NETZSCH STA 449 F3 Jupiter instrument was utilized for the TG – DSC analysis of the compound in the range 25 - 1400° C with the initial sample weight of 4.28 mg in nitrogen atmosphere with nitrogen flow rate 20 ml/min. The graph is plotted by using exothermic in downward direction. A frequency-doubled, Q-switched Nd:YAG (Spectra-Physics, INDI 40) laser, delivering 6 ns laser pulses at 1064 nm at a repetition rate of 10 Hz passed through the powdered form of the title material and their output intensity was measured for screening the second harmonic generation efficiency of the MNMC compound.

Preparation of Resazurin dye solution

The Resazurin dye solution was made by dissolving 270 mg tablet in 40 mL of sterile distilled water. The instrument vortex mixer was used to ensure the formation of homogeneous resazurin solution.

Preparation of the activity plates

96 wells plates were prepared under aseptic conditions. A volume of 200 µL of compounds (1mg/mL) in 5% (v/v) di methyl sulfoxide was pipetted into the first row of the sterile 96 wells plate. 100 µL of nutrient broth was added to other wells for the bacteria cells and 100 µL of Sabouraud dextrose broth for fungus cells. The serial dilutions were performed with sterile pipette tips such that each well had 100 µL of the test material in serially descending concentrations and then 10 µL of resazurin dye solution was added to all these wells. A 10 µL of bacterial suspension (5×10^6 cells/mL) was added to each well to achieve a concentration of 5×10^5 cells/mL. The commercial antibiotic streptomycin (against bacteria) and amphotericin B (against fungus) were used as positive controls in the assay plate. The plates were placed in an incubator at 37° C for 18–24 h. The colour change was then observed visually. The colour changes from purple to pink or colourless were recorded as reduction of dye by the viable bacteria. The lowest concentration at which no colour change occurred was taken as the MIC value.

Protein, ligand preparation and Induced fit docking

The protein human estrogen acceptor data were downloaded from the Protein Data Bank (PDB id: 2IOK). The water molecules were removed and side chains were fixed using the module protein preparation wizard and energy minimized using Optimized Potentials for Liquid Simulations (OPLS) force field. All computational works were performed on CentOS EL-5 workstation using the molecular modelling software Maestro (Schrodinger LLC 2009, USA). GLIDE-5.5 (Grid-based Ligand Docking with Energetics) performs flexible Induced Fit docking (IFD) between the ligand molecule with a macromolecule, usually a protein. PyMOL software was used for graphical visualization, analyzing hydrogen bond interactions and producing quality images. The crystal structure of the compound was drawn using the software Chemsketch and the energy minimized using the impact minimization. The ligand is prepared as a three dimensional structure of drug like molecule in maestro format. The impact module performs conversions, apply corrections to the structures, generate variations on the structures and optimize the structures. The structures were minimized using impact energy minimization with 1000 cycles of steepest descent and 5000 cycles of conjugate gradient.

Induced fit docking study was carried out for the MNMC compound and compared with the co-crystal ligand.

Results and Discussion

FT-IR and FT-RAMAN spectral analysis

FT-IR and FT-RAMAN spectra give information about the vibrational modes of the MNMC compound which aid in the identification of the functional group. The FT-IR and FT-RAMAN spectra of the MNMC compound are shown in Fig. 3a & 3b. The assignment of the IR and Raman bands along with their respective vibrational modes are listed in Table 2.

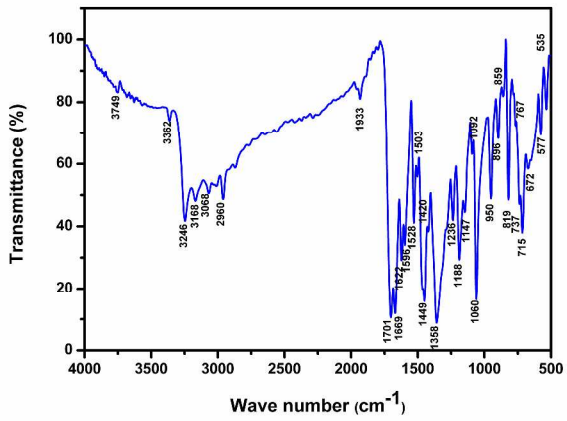


Figure 3a. FT-IR Spectrum of MNMC compound

The weak band at 3749 cm^{-1} in IR and medium peak at 1239 cm^{-1}, 1195 cm^{-1} in Raman are assigned to aromatic O – H stretching. The symmetric stretching of N – H band in IR is observed at 3362 cm^{-1} and 3246 cm^{-1}. The weak peak at 3068 cm^{-1} and 3246 cm^{-1} in IR and the medium peak at 3077 cm^{-1} in Raman correspond to the aromatic C – H stretching of the molecule. The C = O symmetric stretching vibration is found as a very high intense peak at 1701 cm^{-1} in IR and as a weak band at 1623 cm^{-1} and 1669 cm^{-1} in Raman. The medium peak in IR at 1669 cm^{-1} is assigned to C = N symmetry stretching whereas in Raman the stretching represented as a medium and a very high intense peak at 1604 cm^{-1} and 1576 cm^{-1}, respectively.[15-17]

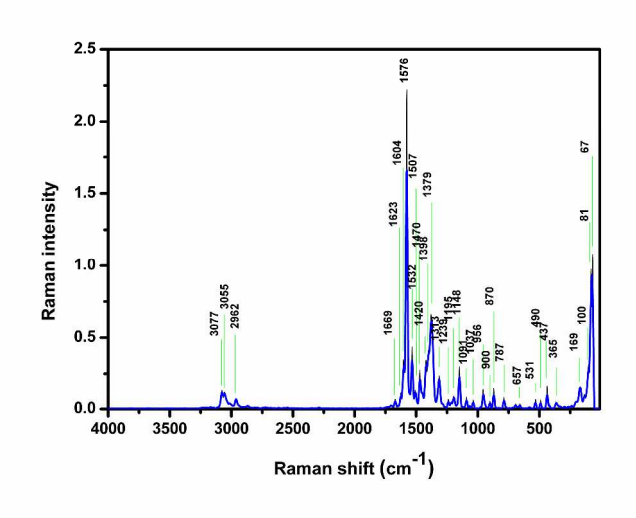


Figure 3b. FT-RAMAN Spectrum of MNMC compound

^{1}H NMR studies

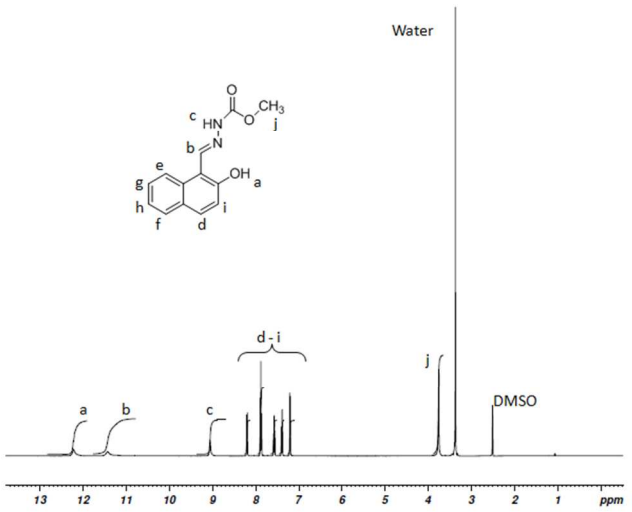


Figure 4. FT – NMR spectrum of MNMC compound

^{1}H NMR spectra give information about the different types of protons in the molecule and also provide the nature of immediate environment to each of them in the molecule. The ^{1}H NMR spectral data for MNMC compound was recorded using DMSO as solvent (Fig. 4). The proton peaks of methoxy group appeared at 3.7 ppm. With different multiplicity and coupling constants, doublet downfield peaks of the aromatic region occur between 7.2 and 8.2 ppm. The azomethine proton undergoes a significant shift at 11.4 ppm as a singlet indicating the coordination of the azomethine nitrogen. Moreover, the singlet at 9.0 ppm confirms the N – H proton of the MNMC molecule. The OH peak shifted towards 12.2 ppm due to its interaction with the azomethine nitrogen.

Ultra Violet-Visible–Diffused Reflectance Spectroscopy (UV-VIS-DRS) studies

The absorption of UV light by a molecule depends on its electronic structure. Hence, the UV spectrum reveals the presence of specific bonding arrangements in the molecule. UV–VIS-DRS was employed for acquisition of the absorption spectra of MNMC (Fig. 5).

There are three main absorption bands centered at 222, 360, 450 nm. The maximum absorption peak was found at 360 nm with cut – off wavelength 405 nm. The $\pi - \pi^*$ transition of C = N chromophore, highly conjugated naphthalene group causes the absorption at 222, 360 nm, respectively, in the near UV region. The absorption band in the visible region arose at 450 nm as a result of the n – π^* transitions of the C = N chromophore.[18,19] As there is no absorption observed in the Nd:YAG laser second harmonic generation wavelength (532 nm), the material can be utilized in the optical application above 500 nm. The study of the absorption edge is essential in connection with the theory of electronic, which leads to the prediction whether the band structure is affected near the band extreme. The most direct way of extracting the optical band gap is to simply determine the photon energy at which there is sudden increase in the absorption. Using the relation $E_g = {}^{hc}/_{\lambda}$, the optical band gap value of MNMC was calculated from the maximum absorption edge as 3.0 eV.

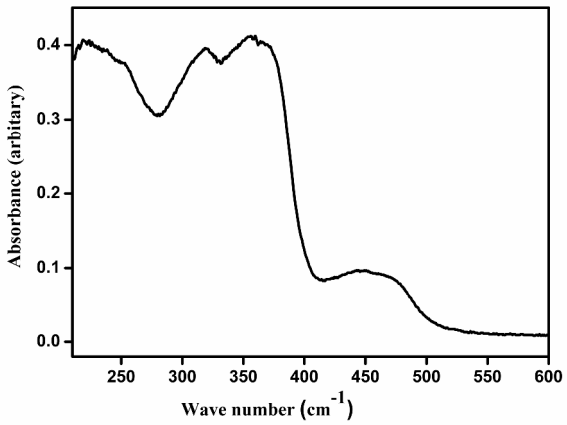


Figure 5. The absorption spectrum of MNMC compound

Cyclic voltammetry

Cyclic voltammetry measurement was carried to measure the HOMO – LUMO energy levels and electrochemical bandgap value. The HOMO – LUMO energy levels of the MNMC compound can be calculated from the electrochemical data by utilizing the equations 1 & 2.[20] The onset oxidation potential $[E_{Ox}(onset)]$ 0.529 V and the onset reduction potential $[E_{Red}\,(onset)]$ - 0.687 V were analysed from cyclic voltammogram (Fig.6).

$$HOMO = -[4.65\,V - E_{Ox}(onset)] = \text{-4.121 eV} \quad (1)$$

$$LUMO = -[4.65\,V - E_{Red}(onset)] = \text{-5.337 eV} \quad (2)$$

The electrochemical bandgap value is calculated from the HOMO and LUMO energy levels using the formula E_g = LUMO – HOMO as 1.2 eV. We have arrived at lesser

energy bandgap value from cyclic voltammogram when compared with the optical bandgap value calculated from UV-VIS-DRS Spectrum.

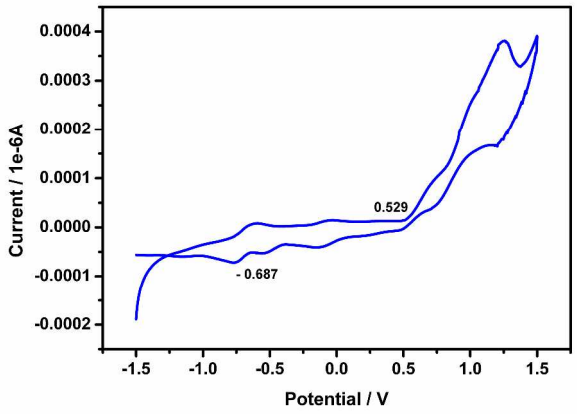


Figure 6. Cyclic voltammogram of MNMC compound

Fluorescence studies

The fluorescence spectrum of MNMC was recorded at room temperature. MNMC compound was excited at wavelength of 365 nm. A broad emission peak at 495 nm (2.5 eV) indicates bluish- green fluorescent emission in MNMC crystal (Fig. 7). The fluorescent peak is obtained at photon energy which is less than the effective band observed by absorption peak and the peak shifts towards lower energy. The emission peak exhibits inhomogeneous broadening (Gaussian distribution) which indicates the formation of defect levels in the material.[21]

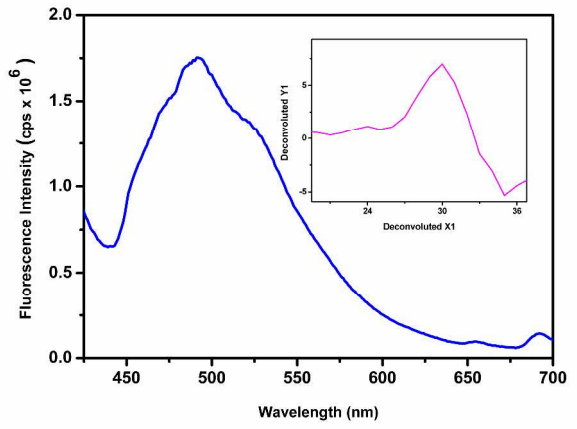


Figure 7. The fluorescent emission spectrum of MNMC (inset: deconvoluted plot)

Antimicrobial studies

The MIC of the compound was determined by resazurin dye reduction assay method described by Sarker *et al*.[12] The color change of the dye from blue or purple to pink indicates that the cells are viable. The enzyme oxido-reductase present inside the bacterial cells or the unicellular fungus convert the resazurin to resorufin which is pink in color. If the color of the dye remains blue then it indicates that there is no activity of viable cells. The compound destroys the bacteria and fungal cells after the incubation period. This was inferred from the color change in respective wells. The pink color change in the wells even after treating with test compounds or commercial drug indicates the presence of viable cells. Thus the least dilution in which the color remains blue was taken as the MIC value of the particular compound.

The test compound was active against all tested human bacterial and fungal pathogens. The compound shows MIC values between 0.78 µg/mL and 25 µg/mL. The compound was effective against the Gram positive bacteria *Streptococcus faecalis and Bacillus cereus* than Gram negative bacteria *Shigella dysenteriae* and *Vibrio cholera* (Fig. 8a). The MIC values of the The title compound is equipotent to reference antibiotic Streptomycin with the MIC value of 1.56 µg/mL against the Gram positive bacteria *Streptococcus faecalis*. In the antifungal study (Fig. 8b), the test compound was more active against the *Candiaglabrata* than the other two fungi *Candida krusei, Candida albicans*. The test compound was less active than the positive control amphotericin B (reference drug) for all the tested fungus. The results are tabulated in Table 3. Thus the given test compound has very good antimicrobial activity. Further development of this compound may have better lead in the biological applications.

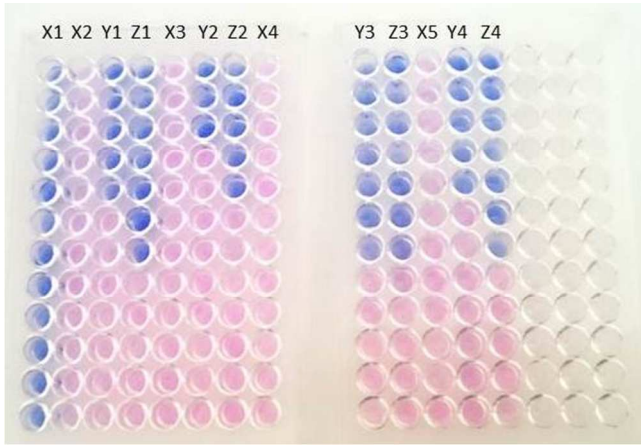


Figure 8a MIC of compound against bacteria by resazurin reduction assay

X1- control- Compound + reagent + without bacteria

X2- control - reagent +*Shigella dysenteriae* + without compound

X3- control - reagent +*Vibrio cholerae* + without compound

X4 -control - reagent + *Streptococcus faecalis* + without compound

X5- control- reagent + *Bacillus cereus*+ without compound

Y1 – Compound + reagent + *Shigella dysenteriae*

Y2– Compound + reagent + *Vibrio cholerae*

Y3 – Compound + reagent + *Streptococcus faecalis*

Y4 – Compound + reagent +*Bacillus cereus*

Z1 –Streptomycin + reagent +*Shigella dysenteriae*

Z2 -Streptomycin + reagent + *Vibrio cholerae*

Z3 –Streptomycin + reagent + *Streptococcus faecalis*

Z4– Streptomycin + reagent + *Bacillus cereus*

Table 3. Minimum Inhibitory Concentration of test compound against microbial pathogens by resazurin reduction assay

MICROBIAL PATHOGENS	MIC µg/ml	
	Compound	Streptomycin
Shigella dysenteriae	6.25	1.56
Vibrio cholerae	25.0	6.25
Streptococcus faecalis	1.56	1.56
Bacillus cereus	6.25	1.56
Candida krusei	12.5	1.56 (amphotericin B)
Candida albicans	25	1.56 (amphotericin B)
Candida glabrata	6.25	0.78 (amphotericin B)

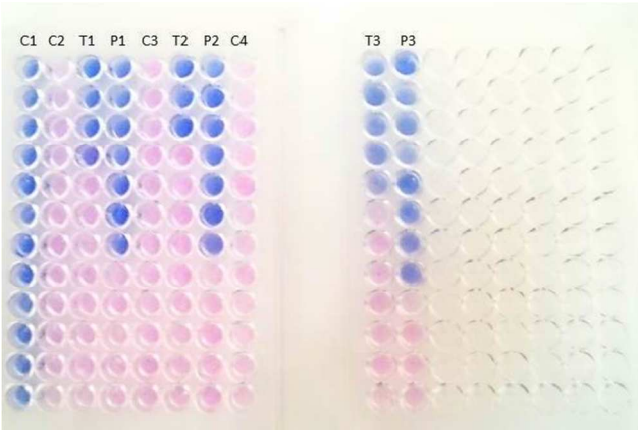


Figure 8b. MIC of compound against fungi by resazurin reduction assay

C1 – Control- Compound + reagent + without fungus

C2– Control - reagent + *Candida krusei* + without compound

C3 – Control - reagent + *Candidaalbicans*+ without compound

C4 – Control - reagent + *Candida glabrata* + without compound

T1- Compound + reagent + *Candida krusei*

T2- Compound + reagent + *Candidaalbicans*

T3- Compound + reagent + *Candida glabrata*

P1- amphotericin B + reagent + *Candida krusei*

P2- amphotericin B + reagent + *Candida albicans*

P3- amphotericin B + reagent + *Candida glabrata*

Molecular docking

Molecular docking is an effective tool to get an insight into the ligand - receptor interactions and to screen molecules for the binding affinities against a particular receptor.[23] Molecular docking study has shown that the compound bound well at the active site of human estrogen receptor. Table 4 represents the induced fit docking studies of the compound with the human estrogen receptor. The oxygen atom of the co-crystal ligand interacts with the oxygen atom of the residue THR347 and the oxygen atom of GLU353 at a distance of 3.0 Å, 2.7 Å, respectively with the glide score of -9.33 and glide energy of -59.95 kcal/mol (Fig. 9a).

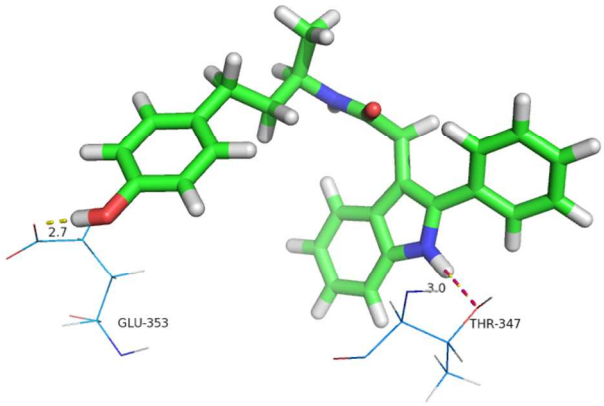


Figure 9a. Interactions of Cocrystal ligand (IOK) at the active site residues

In the MNMC compound under study, the oxygen group interacts with the O_2 atom of the GLU353 and nitrogen group interacts with another O_2 atom of the residue GLU353 at a distance of 3.1 Å and 2.8 Å, respectively. The two O_2 atoms of the compound interact with the residue LYS449 at a distance of 3.0 Å and 3.2 Å, respectively with the glide score of -6.05 and glide energy of -38.90 kcal/mol (Fig. 9b).

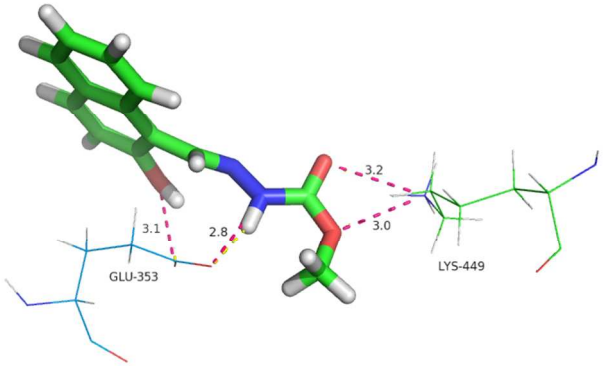


Figure 9b. Interactions of compound Schiff base at the active site residues

Table 4: Induced fit docking studies of the compound with the Human estrogen receptor

Compound	H-Bond Interaction D-H…A	Distance (Å)	Glide Score	Glide Energy (KCal/ Mol)
IOK (cocrystal)	N-H…O(THR 347) O-H…O(GLU 353)	3.0 2.7	-9.33	-59.95
Compound	O-H...O(GLU 353) N-H...O(GLU 353) (LYS449)N-H...O (LYS449)N-H...O	3.1 2.8 3.0 3.2	-6.05	-38.90

Conclusion

Methyl 3–[(E)–(2–hydroxy–1-napthyl) methylidene] carbazate single crystal was grown by employing slow evaporation solution growth technique. The cell parameters were analysed by the single crystal X-ray diffraction analysis and powder X-ray diffraction pattern of the title compound correlated with reported results. The spectral analyses of the compound were performed using FT-IR, FT-RAMAN and Proton NMR confirmed the formation of the title compound. The TG-DTA–DSC study reveals the purity of the compound with the sharp melting of the compound at 205° C and it exhibit two stages of weight loss left with 12.27% residue of mass. From the UV-DRS studies, the band gap value was found to be 3.0 eV corresponding to the UV absorption edge of 405 nm with the maximum absorption peak at 360 nm. The electrochemical bandgap value was calculated as 1.2 eV from cyclic voltammetry measurements which is lesser than optical bandgap value. MNMC showed bluish green emission with a broad peak emission at 495 nm under 365 nm of excitation. Hence the title compound can be utilised for fluorescence applications. The title compound has very good antimicrobial activity against human pathogenic bacteria and fungi. The title compound forms a stable complex with human estrogen receptor which is evident from the ligand –receptor interaction and it may be an effective inhibitor of human estrogen receptor if further biological studies are carried out in the compound in consideration.

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Notes and references

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