Research Article

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Design, Synthesis and Characterization of Novel Isoxazole Tagged Indole Hybrid Compounds

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Abstract: Sixteen new isoxazole tagged indole compounds have been synthesized *via* copper (I) catalyzed click chemistry of the aryl hydroxamoyl chloride and an indole containing alkyne moiety. The chemical structure of the synthesized compounds has been established using various physicochemical techniques. X-ray single crystal analysis of Ethyl 1-((3-phenylisoxazol-5-yl) methyl)-1*H*-indole-2-carboxylate **(8a)** has been analyzed. All compounds were tested for their antibacterial and anticancer activities. The activities for the new compounds were weak against both bacterial strains and the cancer cell lines.

Keywords: isoxazole; indole; click chemistry,1,3-dipolar cycloaddition, Alkyne, Copper (I).

1 Introduction

Indole derivatives, either synthetic or natural, are considered as privileged scaffolds in medicinal chemistry [1–4]. Many of them are the soul of an important class of therapeutic agents, including anticancer [5], antioxidant [6], antirheumatoidal [7], and anti-HIV [8–10] agents. They can also play a vital role in drugs targeting the immune system [11] and as tubulin polymerization inhibitors [12]. In view of the importance of the indole containing compounds, there is an ever-increasing demand to introduce new compounds based on indole moiety.

Isoxazole and their derivatives, on the other hand, have received comparable attention to the indole containing moieties. This is due to their diverse biological activities, which include, but are not limited to, their role as antiplatelet agents [13], antiviral and anti-HIV [14,15], anti-diabetic [16] anti-Alzheimer, anti-cancer and anti-inflammatory agents [17,18].

The nature and the fast pace of chemical research forced scientists to cope by creating reaction conditions that subjugate to its nature. Hence, the relatively new "click chemistry" reactions have been originated by Sharpless and coworkers in 2001 [19,20]. Many reactions were found to satisfy the "click" criteria, the most notable of which are the cycloaddition reactions [21]. The superiority of the click methodology over the traditional Huisgen thermal process is the introduction of metal catalysts such as Cu(I), with the possibility of using other metal catalysts (Ru, Ni, Pt, Pd), within the reaction system at room temperature, rather than resorting to elevated temperatures. This introduction led to an enhancement of the regioselectivity of the product with respect to the Huisgen thermal process [22].

Inspired by the above facts, and in pursuit of our research group theme, which targets research in the realms of drug design and discovery, we report herein, the synthesis of new hybrid scaffolds combining indole and substituted isoxazole based on the well-established "click chemistry" methodology. We are hereby successfully reporting potent antimicrobial agents based on the indole moiety hybridization with other heterocyclic systems, such as triazine [23] and imidazole [24]. The latter-mentioned scaffolds have been reported to be active against various bacterial strains as well as cancer cell lines. (Figure 1)

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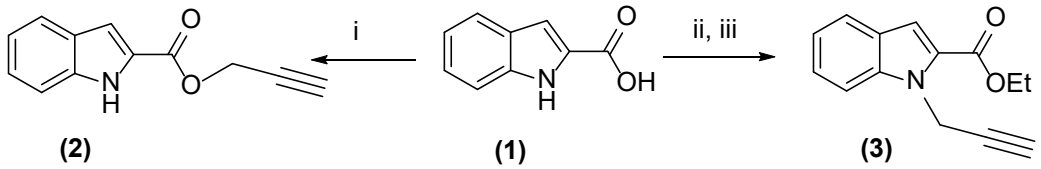
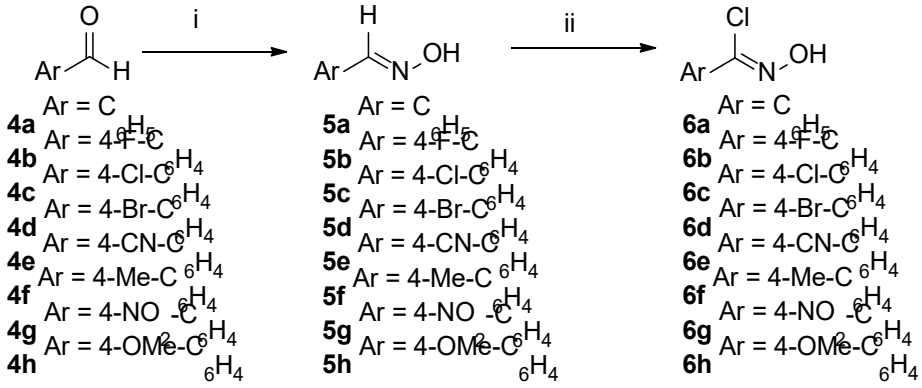


Scheme 1: Synthesis of the click precursors **(2)** and **(3)**; i- Propargyl bromide, K_2CO_3/DMF, 0°C., ii- EtOH/H_2SO_4 reflux 4h's. iii- Propargyl bromide, NaH/DMF, 0°C.



Scheme 2: Synthesis of second click precursor the hydroxamoyl chloride **6a-h**; i- Hydroxylamine hydrochloride, Ar-aldehyde, H_2O/EtOH, 50 % NaOH, 1h, HCl (conc.). ii- oxime, DMF/NCS 2-6 h's.

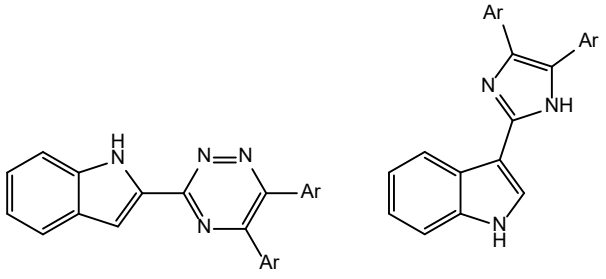


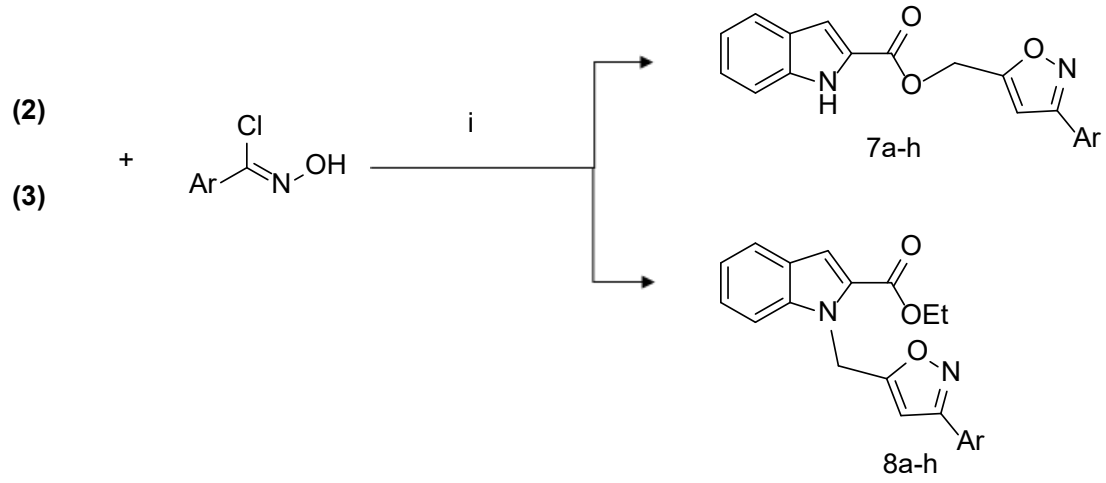
Figure 1: Structure of indole hybridized with triazine **(I)** and imidazole **(II)**.

2 Results, Experimental Procedure, and Discussion

The newly-designed compounds were synthesized from the commercially available 1-*H*-indole-2-carboxylic acid **(1)**. In detail, we utilized both the carboxylic acid and the NH of the indole moiety to introduce our click precursors **(2)** and **(3)**, as shown in Scheme 1. The precursor **(2)** was synthesized in a quantitative yield through the reaction with propargyl bromide in the presence of potassium carbonate in dry DMF. For precursor **(3)** the carboxylic acid group was protected with ethyl ester before reacting it with sodium hydride in dry DMF, and thereafter with propargyl bromide. Both ^{1}H and ^{13}C-NMR analyses for **(2)** and **(3)** clearly depict the characteristic features of the propargyl moiety along with the indole part.

Having the first click precursor in hand, the second precursor was synthesized *via* a two-step process. Initially, a selected set of aldehydes **(4a-h)** were condensed with hydroxyl amine [25]. This was followed by a reaction with N-chlorosuccinimide in dry DMF to give the pure hydroxyimoyl chloride **(6a-h)**, as shown in Scheme 2.

1,3-dipolar cycloaddition was then realized with the dipolarophile **(2)** and **(3)** with the nitrile oxide generated in *situ* from **(6a-h)** upon the action of base directly before the reaction. This will serve to prevent dimerization to furoxans. Clicking both precursors shown in Scheme 1 produced the desired new compounds **(7a-h**) and **(8a-h)** (Scheme 3). All the new compounds were fully characterized using ^{1}H, ^{13}C and 2D-NMR techniques along with HRMS (ESI). In series **(7)** the ^{1}H-NMR shows a clear singlet for 2H resonating around δ = 6.0 ppm assigned for the methylene CH_2 protons, where a 0.5-0.7 down field shift were observed for all the synthesized compounds from the propargylated starting compound **(2)**. Additionally, the new singlet signal assigned for CH in the isoxazole appeared around 6.9-7.5 ppm which was correlated with the signal for the carbon at 100-112 ppm in the ^{13}C-NMR



Scheme 3: Synthesis of compound **(7a to h)** and **(8a to h)**; i- CuI, toluene/Et_3N overnight.

spectrum. In the other series, the compounds show two CH_2 signals, the first signal belongs to the methylene protons of the ester (OCH_2CH_3) at about 4.3 ppm, while the second one belongs to the methylene protons of the carbon attached to indole nitrogen (NCH_2-isoxazole) at about 6.0 ppm. It was also observed that the CH of the isoxazole resonated in the same region as in the first series for both proton and carbon.

HRMS-ESI analysis gave exact molecular ions for all the compounds. The structures of the new derivatives were determined from their corresponding ^1H and ^{13}C-NMR spectra. The formation of the isoxazole moiety was characterized *via* the methine proton signal, that resonates as a singlet at δ = 7.07-7.43 ppm. The singlet at δ = 7.39 ppm was assigned to H-3 of the indole moiety. All compounds show the features of a para-substituted aromatic system along with the aromatic signals of the indole. ^{13}C-NMR, on the other hand, clearly indicates all the features of the synthesized compounds. HMQC, HMBC and DEPT experiments allow us to fully assign all protons and carbons for the new compounds. The NH protons in compounds **(3)** and **(7a-h)** of the indole moiety appeared at 11.90-12.00 ppm for all the synthesized compounds.

X-ray structure determination was performed to further confirm the indole- hybrid system, with compound **(8a)** selected as a representative example of this new class of hybrid system (Figure 2). The molecule crystallizes in a monoclinic chiral space group $P2_1$. A summary of data collection and refinement parameters are given in Table 1. Figure 2 of compound **(8a)** shows that the indole along with the carboxylate moieties is in one plane with the CH2-isoxazole plane. Additionally, the isoxazole ring is connected to the indole via the CH_2 moiety with angle N1C12C13 of 110.7(3)°. An insight into intermolecular interactions can be obtained by electrostatic potential map and two-dimensional fingerprint plots mapped in the Hirschfeld surface, analyzed by CrystalExplorer 17.5 [26–28]. The electrostatic potential of compound **(8a)** (Figure 3a) shows that the isoxazole plane is almost negative towards the oxygen atoms while the indole plane is positive. This makes the molecule neutral with non-favorable intermolecular interactions. The two dimensional fingerprint plots and the contributions of individual interatomic contacts toward the overall crystal packing are shown in Figure 3b. Several directional contacts can be observed, such as C–C (1.8%), C–H (16.4%), N–H (4.2%), O–H (7.8%) along with H–H (66.7%). These intermolecular atom-atom contacts contribute to the stability of the crystal packing of compound **(8a).**

Ethical approval: The conducted research is not related to either human or animal use.

2.1 Biological Activities

Indole derivatives have shown various antibacterial and anticancer activities. In regards to the antimicrobial activity, this study reported weak *in vitro* antibacterial activity against both Gram positive and Gram negative bacteria. In addition, no significant activity was reported against pathogenic yeast and fungi. By the initial screening against selected pathogenic bacteria including *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, the minimal inhibitory concentrations of the tested compounds were higher than 1 mg / ml.

For the anticancer activity testing of the synthetic compounds, the inhibitory concentrations and percentage of killing of the tested compounds were measured using three human cancer cell lines: Leukemic cells (Hela), breast cancer cells (MCF-7), and epiglottis cancer cells (Hep-2) in addition to a non-human Chinese hamster ovary (CHO) cell lines. A concentration range of 100-500 ug/mL was used for all cells, and cell viability assay MTT was performed. A weak anticancer activity was observed for the tested compounds. Furthermore, Hela cell lines showed the highest sensitivity to all chemicals (% of killing equals to 20-34), whereas Hep-2 and MCF-7 cells were the least sensitive (% of killing equals to 5-11).

2.2 Experimental

^{1}H-NMR spectra were recorded in hexadeuterodimethyl sulfoxide (DMSO-d_6) and deuterated chloroform ($CDCl_3$) on a Bruker Avance III-300 MHz and III-500 MHz Spectrometer with TMS as an internal standard. ^{13}C-NMR spectra were obtained at 75 MHz and 125 MHz on the same instruments, respectively. Chemical shifts were reported as δ-values in ppm. High resolution Mass spectra (HRMS) were recorded in positive ion mode by Electro Spray Ionization (ESI) using a Bruker Daltonics Apex IV, 7.0 T Ultra Shield Plus. The samples were dissolved in chloroform, diluted in spray solution (methanol / water 1:1 v/v + 0.1% formic acid) and infused using a syringe pump with a flow rate of 2 µL / min. External calibration was conducted using arginine cluster in a mass range m/z 175-871. For all HRMS data, mass error falls in the range of 0.00–0.50 ppm. Melting points (m.p.) were determined on an Electrothermal Melting Point Apparatus and were uncorrected in °C. Solvents used in this study were obtained from Scharlau, Fluka, Acros, and Aldrich. All reactions were monitored by thin layer chromatography (TLC) using Merck aluminum plates pre-coated with silica gel PF254; 20 × 20 × 0.25 mm, and detected by visualization of the plate under UV lamp (λ = 254 or 365 nm). Spots were also detected by spraying with anisaldehyde- sulphuric acid in ethanol, followed by heating to 140°C [35].

2.2.1 X-Ray Crystallography

Compound **(8a)** was recrystallized from hot DMF/benzene after cooling to room tempetaure. Small amount of ice was added to produce a fine colorless block crystalline solid. The compound crystallized in monoclinic chiral space group $P2_1$. A summary of the crystallographic data and structure refinement parameters is given in Table 1.

Table 1: Crystal data and structure refinement for compound **8a.**

Empirical formula	$C_{21}H_{18}N_2O_3$
Formula weight	346.37
Appearance	Block, colorless
Crystal system	Monoclinic,
Space group	$P2_1$
Temperature	291 K°
A/å	6.7129 (4)
B/å	12.5657 (8)
C/å	1610.8955 (9)
β/°	104.802 (7)°
Volume/å^3	888.57 (11)
Z	2
P_{calc}mg/mm^3	1.295
µ/mm^{-1}	0.09
F(000)	364
Θ range for data collection	3.2–29.4°
Index ranges	-29 ≤ h ≤ 27, -28 ≤ k ≤ 29, -21 ≤ l ≤ 10
Reflections collected	4257
Independent reflections	2979 [R(int) 0.017]
Data/restraints/parameters	3501/0/209
Goof on f^2	1.063
Final r indexes [i>=2σ (i)]	R_1 = 0.038 , wR_2 = 0.1029
Final r indexes [all data]	R_1 = 0.0487, wR_2 = 0.1097*
Largest diff. Peak/hole/eå$^{-3}$	3.08 /-1.28

Single-crystal X-ray diffraction data were collected using an Oxford Diffraction XCalibur, equipped with (Mo) X-ray Source (λ = 0.71073 Å) at room temperature (293(2) K). Data collection, reduction, and cell refinement were performed using the software package CrysAlisPro [29]. Analytical absorption corrections were applied using spherical harmonics implemented in SCALE3 (ABSPACK) scaling algorithm. Crystal structure was solved by direct methods, using the program OLEX2, followed by Fourier synthesis, and refined on F2 with SHELXL implemented in OLEX2 [30,31]. Anisotropic least-squares refinement of non-H atoms was applied. All crystallographic plots were obtained using the CrystalMaker program [32].

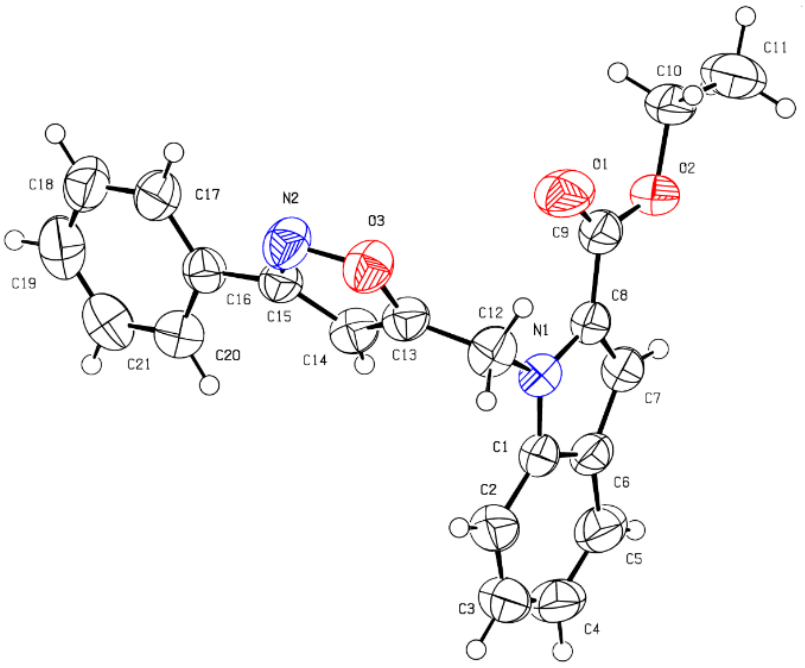


Figure 2: Molecular structure and atom numbering scheme of **8**a. Thermal ellipsoids are drawn at 30% probability level.

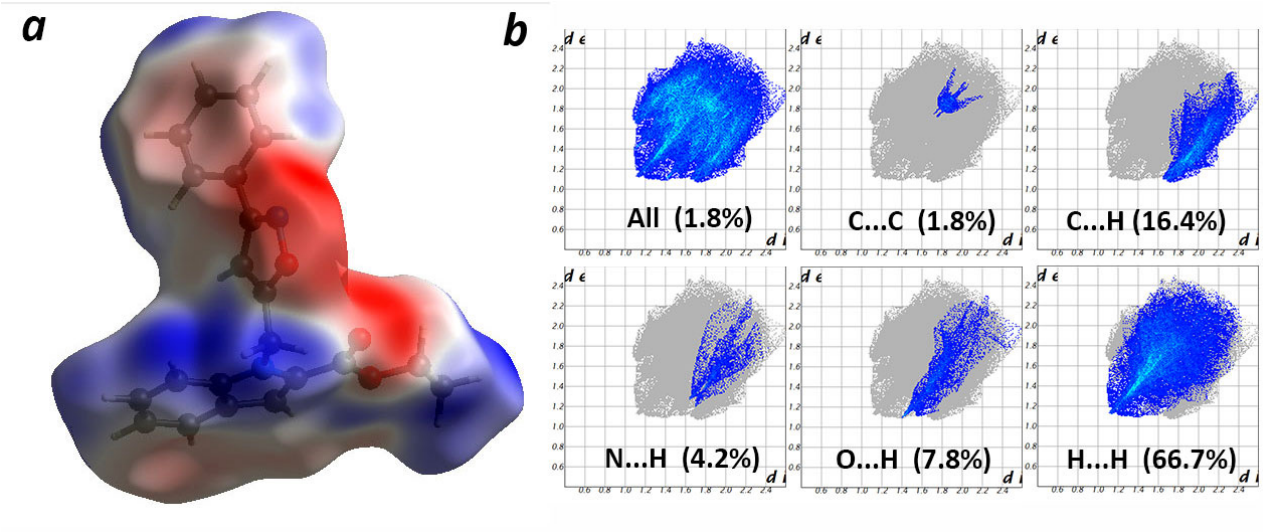


Figure 3: a) Compound **(8a)** mapped over the Hirschfeld surface with a scale of -0.07 a.u. (red) through 0.0 (white) to +0.04 a.u. (blue); b) Two-dimensional full fingerprint plots and decomposed fingerprint plots over the Hirschfeld surface for various intermolecular atom–atom contacts in compound **(8a)**. The numbers in brackets indicate the percentage contributions of each contact.

2.2.2 Prop-2-yn-1-yl 1H-indole-2-carboxylate (2)

1H-indole-2-carboxylic acid (1) 1.0 g (6.20 mmol) was dissolved in 10 ml DMF, and 5 equivalent of K_2CO_3, 4.28 g (31 mmol), were added at 0°C. After 30 minutes, propargyl bromide (1.5 ml, 19 mmol) was added to the reaction mixture. After two hours, TLC confirmed the completion of the reaction; which was poured over crushed ice. The white precipitate that formed was collected and recrystallized from hot ethyl acetate. The product was produced with a high yield of 90.0%, and a melting point range of 136-138°C.

2.2.3 .Ethyl-1-(prop-2-yn-1-yl)-1H-indole-2-carboxylate (3)

1-H-indole-2-carboxylic acid (1) was transformed into ethyl 1-H-indole-2-carboxylate using general method of esterification, where the indole acid was refluxed in ethanol in the presence of concentrated sulfuric acid (H_2SO_4) to produce indole ester with a high yield of 93.5% as a white solid after work up with water. The reaction of the ethyl 1-H-indole-2-carboxylate (3) with propargyl bromide was performed under an inert gas mixture (N_2, Ar) in the presence of sodium hydride (NaH) and DMF as a solvent, to produce the ethyl-1-(prop-2-yn-1-yl)-1H-indole-2-carboxylate as a white solid. This white solid was then recrystallized from hot EtOH. The product was obtained with a high yield of 80.7% and melting point range of 65-67°C.

2.2.4 General Procedure for the Synthesis of Aryl Oximes (5a-h)

The designed oximes were prepared according to the general procedure described herein. Hydroxylamine hydrochloride (0.55 mol) was added to a mixture of aryl aldehyde (5a-h) (0.25 mol) dissolved in 30 ml H_2O/EtOH (1:1) and 30 ml of ice. Afterwards, 0.63 mol of 50.0% NaOH was added dropwise and the reaction was stirred at room temperature for approximately one hour, and monitored by TLC in chloroform ($CHCl_3$). After completion of the reaction, neutralization was accomplished using a concentrated HCl solution. Solid oximes were collected by suction filtration while liquid oximes were extracted with $CHCl_3$ (20mL×2), dried over $MgSO_4$. The solvent was evaporator; the aryl oximes were produced with high yields (74 – 93%) [27].

2.2.5 General Procedure for the Synthesis of Hydroxamoyl Chloride (6a-h)

To a stirred solution of 1.0 g of oxime (6a-h) in 15 ml DMF at room temperature, 1.3 mol equivalent of NCS was added. The initial addition (about 1/10 of the mass) results in a slight increase of temperature. In case that does not happen, then the solution is heated to 45°C to initiate the reaction. Once the reaction is initiated, the rest of NCS was added portion wise and the temperature was kept under 35°C. The mixture was stirred for 3-6 hours, and monitored by TLC (in $CHCl_3$). After completion of the reaction, the mixture was poured over crushed ice and the resultant product was filtrated as a solid. In the case of liquid products, they were extracted using $CHCl_3$ (20 mL×2) dried over $MgSO_4$, and the solvent was removed using rotary evaporator. All hydroxamoyl chloride compounds (**6a-h**) were used in situ directly without further purification.

2.2.6 General Procedure for the Synthesis of Series (7) and (8)

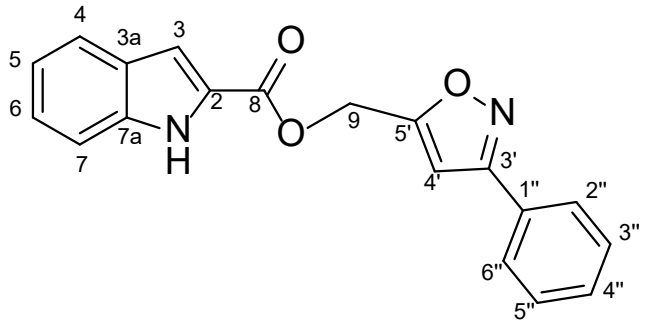
A mixture of compound **3** (1.0 mmol) with 0.019 g copper (I) Iodide, CuI, (10 mmol) in the presence of Et_3N (4.07 mmol) was stirred for 30 minutes. After that, 5-7 ml of toluene was added and the solution was stirred for an additional 30 minutes. To this solution, 1.3 mol equivalent of the corresponding hydroxamoyl chloride (**7a**-**h**) was added. The reaction mixture was stirred for 17-24 h. and monitored by TLC. After completion, 25 ml of EtOAc was added with stirring and the copper salt was removed by filtration. The filtrate was extracted and the combined organic layer was dried over Na_2SO_4. The solvent was evaporated and the product was purified using normal thin layer chromatography with Hexane/ethyl acetate as mobile phase (the ratio depended on the product polarity). Figure 4 shows the numbering system adopted in this study with compounds **7a** and **8a** used as representatives for this order.

(3-phenylisoxazol-5-yl)methyl 1H-indole-2-carboxylate **(7a)**

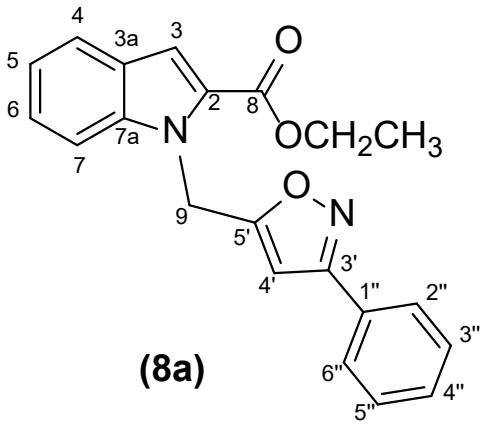
Yield: 57%; white solid; m.p: 196 - 198°C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.53 (s, 2H, H-9), 7.05 (pseudo t, H-5), 7.19 (s, H-4'), 7.24 (s, H-3), 7.25 (pseudo t, H-6), 7.43 (d, J = 8.3 Hz, H-7), 7.47 (m, H-4''), 7.48 (m, H-3'', H-5''),7.65 (d, J = 8.1 Hz, H-4), 7.86 (m, H-2'', H-6''), 11.90 (s, N*H*). ^{13}C NMR (125 MHz, DMSO-d_6): 57.1 (C-9), 103.0 (C-4'), 109.4 (C-3), 113.1 (C-7), 120.8 (C-5), 122.7 (C-4), 125.5 (C-6), 126.6 (C-2), 126.8 (C-3a), 127.1 (C-2'', C-6''), 128.7 (C-1''), 129.6 (C-3'', C-5''), 130.9 (C-4''), 138.2 (C-7a), 161.0 (C-5'), 163.0 (C-3'), 168.2 (C-8). HRMS (ESI) m/z: Calcd. For $C_{19}H_{14}N_2O_3Na$ $[M + Na]^+$ 341.09021. Found: 341.08966.

(3-(4-fluorophenyl)isoxazol-5-yl)methyl *1H*-indole-2-carboxylate **(7b)**

Yield: 52%; White solid; mp: 155 - 157°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 5.58 (s, 2H, H-9), 7.10 (pseudo t, H-5), 7.27 (s, H-4'), 7.29 (s, H-3), 7.29 (pseudo t, H-6), 7.37 (pseudo t, H-2'', H-6''), 7.48 (d, J = 8.3 Hz, H-7), 7.68 (d, J = 8.1 Hz, H-4), 7.99 (dd, J_{H-H} = 8.6 Hz , J_{H-F} = 14.2 Hz, H-3'', H-5''), 11.99 (s, N*H*). ^{13}C NMR (125 MHz, DMSO-d6): δ 57.0 (C-9), 102.9 (C-4'), 109.3 (C-3), 113.1 (C-7), 116.7 (^{2}J C-F = 21.9 Hz, C-3'', C-5''), 120.5 (C-5), 122.4 (C-4), 125.2 (C-6), 126.6 (C-3a), 127.1 (C-2), 129.5 (C-1''), 129.7 (^{3}J C-F = 8.7 Hz, C-2'', C-6''), 138.1 (C-7a), 160.9 (C-5'), 161.6 (C-3'), 163.8 (^{1}J C-F = 247.6 Hz, C-4''), 168.5 (C-8). HRMS (ESI) m/z: Calcd. For $C_{19}H_{12}FN_2O_3$ $[M - H]^+$ 335.08320. Found: 335.08374.



(7a)



(8a)

Figure 4: structure and numbering of compounds **(7a)** and **(8a)**.

(3-(4-chlorophenyl)isoxazol-5-yl)methyl *1H*-indole-2-carboxylate **(7c)**

Yield 54%; White solid; mp: 180 - 182°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.53 (bs, 1H), 7.77 (bd, *J* = 8Hz, 1H), 7.69 (m, 1H), 7.48-7.60 (m, 9H); 7.34-7.37 (m, 1H), 7.19-7.22 (m, 1H). ^{13}C NMR (125 MHz, DMSO-d6): δ 57.0 (C-9), 102.9 (C-4'), 109.4 (C-3), 113.1 (C-7), 120.8 (C-5), 122.6 (C-4), 125.5 (C-6), 126.5 (C-3a), 127.1 (C-2), 127.5 (C-1''), 128.6 (C-3'', C-5''), 129.4 (C-2'', C-6''), 135.5 (C-4''), 138.1 (C-7a), 160.9 (C-5'), 161.7 (C-3'), 168.5 (C-8). HRMS (ESI) m/z: Calcd. for $C_{19}H_{13}ClN_2O_3Na$ [M + Na]$^+$ 375.05124. Found: 375.05069.

(3-(4-bromophenyl)isoxazol-5-yl)methyl *1H*-indole-2-carboxylate **(7d)**

Yield 46%; White solid; mp: 165 - 168°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 5.59 (s, 2H, H-9), 7.10 (pseudo t, H-5), 7.29 (s, H-3), 7.29 (pseudo t, H-6), 7.29 (s, H-4'), 7.48 (d, J = 8.2 Hz, H-7), 7.68 (d, J = 8.0 Hz, H-4), 7.74 (d, J = 8.4 Hz, H-2'', H-6''), 7.87 (d, J = 8.4 Hz, H-3'', H-5''), 11.99 (s, N*H*). ^{13}C NMR (125 MHz, DMSO-d6): δ 57.1 (C-9), 103.0 (C-4'), 109.4 (C-3), 113.1 (C-7), 120.8 (C-5), 122.7 (C-4), 124.3 (C-4''), 125.5 (C-6), 126.5 (C-3a), 127.1 (C-2), 127.9 (C-1''), 129.2 (C-2'', C-6''), 132.6 (C-3'', C-5''), 138.1 (C-7a), 160.9 (C-5'), 161.7 (C-3'), 168.6 (C-8).

(3-(4-cyanophenyl)isoxazol-5-yl)methyl *1H*-indole-2-carboxylate **(7e)**

Yield 34%; White solid; mp: 162 - 164°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 5.63 (s, 2H, H-9), 7.10 (pseudo t, H-5), 7.29 (s, H-3), 7.29 (pseudo t, H-6), 7.40 (s, H-4'), 7.48 (d, J = 8.2 Hz, H-7), 7.68 (d, J = 8.0 Hz, H-4), 8.01 (d, J = 8.2 Hz, H-3'', H-5''), 8.15 (d, J = 8.2 Hz, H-2'', H-6''), 12.00 (s, N*H*). ^{13}C NMR (125 MHz, DMSO-d6): δ 57.1 (C-9), 103.3 (C-4'), 109.4 (C-3), 113.1 (C-7), 113.3 (C-4''), 118.8 (CN), 120.8 (C-5), 122.6 (C-4), 125.5 (C-6), 126.5 (C-3a), 127.1 (C-2), 127.9 (C-2'', C-6''), 133.0 (C-1''), 133.6 (C-3'', C-5''), 138.2 (C-7a), 160.9 (C-5'), 161.4 (C-3'), 169.0 (C-8). HRMS (ESI) m/z: Calcd. For $C_{20}H_{12}N_3O_3$ [M - H]$^+$ 342.08787. Found: 342.08763.

(3-(p-tolyl)isoxazol-5-yl)methyl *1H*-indole-2-carboxylate **(7f)**

Yield 35%; White solid; mp: 165 - 167°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.38 (s, 3H, CH_3), 5.57 (s, 2H, H-9), 7.10 (pseudo t, H-5), 7.21 (s, H-4'), 7.29 (s, H-3), 7.29 (pseudo t, H-6), 7.34 (d, J = 7.9 Hz, H-3'', H-5''),7.48 (d, J = 8.2 Hz, H-7), 7.68 (d, J = 8.0 Hz, H-4), 7.79 (d, J = 7.9 Hz, H-2'', H-6''), 11.90 (s, N*H*). ^{13}C NMR (125 MHz, DMSO-d6): δ 21.4 (CH_3), 57.1 (C-9), 102.8 (C-4'), 109.3 (C-3), 113.1 (C-7), 120.8 (C-5), 122.6 (C-4), 125.5 (C-6), 125.9 (C-2), 126.5 (C-3a), 127.0 (C-2'', C-6''), 127.1 (C-4''), 130.1 (C-3'', C-5''), 138.1 (C-7a), 140.5 (C-1''), 160.9 (C-5'), 162.4 (C-3'), 168.0 (C-8). HRMS (ESI) m/z: Calcd. For $C_{20}H_{15}N_2O_3$ [M - H]$^+$ 333.12392. Found: 333.12337.

(3-(4-nitrophenyl)isoxazol-5-yl)methyl *1H*-indole-2-carboxylate **(7g)**

Yield 22%; Off-white solid; mp: 150 - 153°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 5.62 (s, 2H, H-9), 7.10 (pseudo t, H-5), 7.29 (s, H-3), 7.29 (pseudo t, H-6), 7.43 (s, H-4'), 7.48 (d, J = 8.3 Hz, H-7), 7.68 (d, J = 8.0 Hz, H-4), 8.20 (d, J = 8.7 Hz, H-2'', H-6''), 8.37 (d, J = 8.7 Hz, H-3'', H-5''), 12.00 (s, N*H*). ^{13}C NMR (125 MHz, DMSO-d6): δ 21.4 (CH_3), 57.1 (C-9), 102.8 (C-4'), 109.3 (C-3), 113.1 (C-7), 120.8 (C-5), 122.6 (C-4), 125.5 (C-6), 125.9 (C-2), 126.5 (C-3a), 127.0 (C-2'', C-6''), 127.1 (C-4''), 130.1 (C-3'', C-5''), 138.1 (C-7a), 140.5 (C-1''), 160.9 (C-5'), 162.4 (C-3'), 168.0 (C-8). HRMS (ESI) m/z: Calcd. for $C_{19}H_{13}N_3O_5$ [M - H]$^+$ 362.07770 .Found: 362.7824.

(3-(4-methoxyphenyl)isoxazol-5-yl)methyl *1H*-indole-2-carboxylate **(7h)**

Yield 36%; white solid; mp: 146 - 149°C. 1H NMR (500 MHz, DMSO-d_6): δ 3.83 (s, 3H, OCH_3), 5.56 (s, 2H, H-9), 7.07 (d, J = 8.6 Hz, H-3'', H-5''), 7.10 (pseudo t, H-5), 7.19 (s, H-4'), 7.29 (s, H-3), 7.29 (pseudo t, H-6), 7.48 (d, J = 8.3 Hz, H-7), 7.68 (d, J = 8.0 Hz, H-4), 7.84 (d, J = 8.6 Hz, H-2'', H-6''), 11.99 (s, NH). 13C NMR (125 MHz, DMSO-d6): δ 55.8 (OCH3), 57.0 (C-9), 102.7 (C-4'), 109.3 (C-3), 113.1 (C-7), 115.0

(C-3'', C-5''), 120.8 (C-5), 121.6 (C-1''), 122.6 (C-4), 125.5 (C-6), 126.6 (C-3a), 127.1 (C-2), 128.4 (C-2'', C-6''), 138.1 (C-7a), 160.9 (C-5'), 161.1 (C-3'), 162.1 (C-4''), 167.8 (C-8). HRMS (ESI) m/z: Calcd. for $C_{20}H_{15}N_2O_3$ [M - H]$^+$ 347.10373. Found: 347.10372.

Ethyl 1-((3-phenylisoxazol-5-yl)methyl)-1*H*-indole-2-carboxylate **(8a)**

Yield 60.5%; white solid; mp: 112 – 113°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.33 (t, J = 7.10 Hz, CH_2CH_3), 4.34 (q, J = 7.10 Hz, OCH_2CH_3), 6.08 (s, NCH_2C), 6.79 (s, H-4'), 6.89 (s, H-3), 7.19 – 7.81 (m, H-4, H-5, H-6, H-7, H-2'', H-3'', H-4'', H-5'', H-6''). ^{13}C NMR (125 MHz, DMSO-d_6): δ = 14.6 (CH_2CH_3), 40.4 (NCH_2C), 61.1 (OCH_2CH_3),100.8 (C-4'), 111.6 (C-7), 111.7 (C-3), 121.7 (C-5), 123.1 (C-4), 126.0 (C-6), 126.1 (C-3a), 127.1 (C-2'', C-6''), 127.5 (C-2), 128.7 (C-4''), 129.5 (C-3'', C-5''), 130.7 (C-1''), 139.4 (C-7a), 161.6 (C-5'), 162.3 (Ar-COO), 170.1 (C-3'). HRMS (ESI) m/z: Calcd. for $C_{21}H_{18}N_2O_3^+$ [M+H]$^+$ 347.13902. Found: 347.13903

Ethyl 1-((3-(4-fluorophenyl)isoxazol-5-yl)methyl)-1H-indole-2-carboxylate **(8b)**

Yield 41.2%; white solid; mp: 109 – 110°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.33 (t, J = 6.5 Hz, CH_2CH_3), 4.34 (q, J = 6.5 Hz, OCH_2CH_3), 6.07 (s, NCH_2C), 6.80 (s, H-4'), 7.20 (overlapped dd, J = 7.1, 6.9, H-5), 7.31 (overlapped dd, J = 8.1, 8.2 Hz, H-3'', H-5''), 7.41 (m, H-3, H-6), 7.76 (m, H-4, H-7), 7.87 (m, H-2'', H-6''). ^{13}C NMR (125 MHz, DMSO-d_6): δ = 14.6 (CH_2CH_3), 40.4 (NCH_2C), 61.1 (OCH_2CH_3), 100.8 (C-4'), 111.6 (C-3), 111.7 (C-7), 116.6 (^{2}J C-F = 21.9 Hz, C-3'', C-5''), 121.67 (C-5), 123.1 (C-4), 125.3 (^{4}J C-F = 3.2 Hz, C-1''), 126.0 (C-6), 126.1 (C-3a), 127.4 (C-2), 129.5 (^{3}J C-F = 8.6 Hz, C-2'', C-6''), 139.5 (C-7a), 161.5 (Ar-COO), 161.6 (C-5'), 163.7 (^{1}J C-F = 247.8 Hz, C-4''), 170.3 (C-3'). HRMS (ESI) m/z: Calcd. for $C_{21}H_{17}FN_2O_3^+$ [M+H]$^+$ 387.11154. Found: 387.11145.

Ethyl 1-((3-(4-chlorophenyl)isoxazol-5-yl)methyl)-1H-indole-2-carboxylate **(8c)**

Yield 21.1%; white solid; mp: 117 – 119°C. 1H NMR (300 MHz, DMSO-d_6): δ 1.39 (t, J = 7.1 Hz, CH_2CH_3), 4.37 (q, J = 7.1 Hz, OCH_2CH_3), 5.95 (s, NCH_2C), 6.22 (s, H-4'), 7.19 (overlapped dd, J = 7.8, 7.2 Hz, H-5), 7.34 (d, J = 8.3 Hz, H-3'', H-5'') ,7.39 (m, H-3, H-6), 7.49 (d, J = 8.5 Hz, H-4), 7.62 (d, J = 8.3 Hz, H-2'', H-6''), 7.69 (d, J = 7.8 Hz, H-7). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.4 (CH_2CH_3), 40.2 (NCH_2C), 60.9 (OCH_2CH_3), 100.3 (C-4'), 110.4 (C-3), 111.8 (C-7), 121.5 (C-5), 122.9 (C-4), 125.9 (C-6), 126.2 (C-3a), 127.1 (C-1''), 127.3 (C-2), 128.1 (C-2'', C-6''), 129.1 (C-3'', C-5''), 136.1 (C-4''), 139.2 (C-7a), 161.6 (C-5'), 162.0 (Ar-COO), 169.7 (C-3'). HRMS (ESI) m/z: Calcd. for $C_{21}H_{17}ClN_2O_3^+$[M+H]$^+$ 381.10005. Found: 381.10003.

Ethyl 1-((3-(4-bromophenyl)isoxazol-5-yl)methyl)-1H-indole-2-carboxylate **(8d)**

Yield 52.7%; white solid; mp: 108 – 109°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.27 (t, J = 7.1 Hz, CH_2CH_3), 4.29 (q, J = 7.1 Hz, OCH_2CH_3), 6.02 (s, NCH_2C), 6.77 (s, H-4'), 7.15 (overlapped dd, J = 7.4, 7.7 Hz, H-5), 7.27 – 7.73 (m, 8H, ArH). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 14.6 (CH_2CH_3), 40.4 (NCH_2C), 61.1 (OCH_2CH_3), 100.8 (C-4'), 111.6 (C-3), 111.7 (C-7), 121.7 (C-5), 123.1 (C-4), 124.2 (C-6), 126.1 (C-3a), 127.5 (C-1''), 127.9 (C-2), 129.1 (C-2'', C-6''), 132.6 (C-3'', C-5''), 130.7 (C-4''), 139.5 (C-7a), 161.6 (C-5'), 161.6 (Ar-COO), 170.5 (C-3'). HRMS (ESI) m/z: Calcd. for $C_{21}H_{17}BrN_2O_3Na^+$ [M+Na]$^+$ 447.03148. Found: 447.03236.

Ethyl 1-((3-(4-cyanophenyl)isoxazol-5-yl)methyl)-1H-indole-2-carboxylate **(8e)**

Yield 50.3%; white solid; mp: 154 – 158°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.27 (t, J = 7.1 Hz, CH_2CH_3), 4.29 (q, J = 7.1 Hz, OCH_2CH_3), 5.51 (s, NCH_2C), 6.05 (s, H-4'), 6.89 (s, H-3), 7.16 (pseudo t, J = 7.8 Hz, H-5), 7.27 – 7.74 (m, H-4, H-6, H-7), 7.90 (d, J = 8.4 Hz, H-3'', H-5''), 7.98 (d, J = 8.4 Hz, H-2'', H-5''). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 14.6 (CH_2CH_3), 40.6 (NCH_2C), 61.2 (OCH_2CH_3), 101.2 (C-4'), 111.6 (C-3), 111.8 (C-7), 113.2 (C-4''), 118.9 (C≡N), 121.7 (C-5), 126.1 (C-4), 127.0 (C-6), 127.5 (C-3a), 128.0 (C-2'', C-6''), 133.0 (C-2), 133.5 (C-3'', C-5''), 139.0 (C-1''), 139. 5 (C-7a), 161.3 (C-5'), 161.6 (Ar-COO), 171.0 (C-3'). HRMS (ESI) m/z: Calcd. for $C_{22}H_{17}N_3O_3^+$[M+H]$^+$ 372.13427. Found: 372.13426.

Ethyl 1-((3-(p-tolyl)isoxazol-5-yl)methyl)-1H-indole-2-carboxylate **(8f)**

Yield 59.5%; white solid; mp: 80 – 81°C. ^{1}H NMR (300 MHz, DMSO-d_6) : δ = 1.28 (t, J = 7.1 Hz, CH_2CH_3), 2.29 (s, Ar-CH_3), 4.29 (q, J = 7.1 Hz, OCH_2CH_3), 6.01 (s, NCH_2C), 6.68 (s, H-4'), 7.13 (overlapped dd, J = 7.4, 7.3 Hz, H-5), 7.22 (d, J =7.8 Hz, H-3'', H-5''), 7.30 – 7.43 (m, H-3, H-6), 7.64 (d, J = 7.8 Hz, H-2'', H-6''), 7.70 (d, J = 7.8, H-4, H-7). ^{13}C NMR (75 MHz, DMSO-d_6) : δ = 14.8 (CH_2CH_3), 21.4 (Ar-CH_3), 40.5 (NCH_2C), 61.1 (OCH_2CH_3), 100.7 (C-4'), 111.6 (C-3), 111.7 (C-7), 121.7 (C-5), 123.1 (C-4), 125.9 (C-2), 126.1 (C-6), 127.0 (C-2'', C-6''), 127.5 (C-1''), 130.1 (C-3'', C-5''), 139.5 (C-7a), 140.5 (C-4''), 161.6 (C-5'), 162.3 (Ar-COO), 169.9 (C-3'). HRMS (ESI) m/z: Calcd. for $C_{22}H_20N_2O_3^+$[M+H]$^+$ 361.15467. Found: 361.15450.

Ethyl 1-((3-(4-nitrophenyl)isoxazol-5-yl)methyl)-1H-indole-2-carboxylate **(8g)**

Yield 57.4%; green solid; mp: 111 – 114°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.32 (t, J = 7.0 Hz, CH_2CH_3), 4.34 (q, J = 7.0 Hz, OCH_2CH_3), 6.11 (s, NCH_2C), 6.96 (s, H-4'), 7.21 (overlapped dd, J = 6.9, 7.7 Hz, H-5), 7.39 (m, H-3, H-6) ,7.77 (m, H-4, H-7), 8.11 (d, J = 7.4 Hz, H-2'', H-6''), 8.31 (d, J = 7.4 Hz, H-3'', H-5''). ^{13}C NMR (125 MHz, DMSO-d^6) : δ = 14.6 (CH_2CH_3) , 40.6 (NCH_2C), 61.1 (OCH_2CH_3), 101.3 (C-4'), 111.5 (C-3), 111.8 (C-7), 121.7 (C-5), 123.1 (C-4), 124.7 (C-3'', C-5''), 126.1 (C-6), 127.4 (C-2), 128.4 (C-3a), 128.8 (C-2'', C-6''), 134.7 (C-1''), 139.4 (C-7a), 148.8 (C-4''), 161.0 (C-5'), 161.6 (Ar-COO), 171.1 (C-3'). HRMS (ESI) m/z: Calcd. for $C_{21}H_{17}N_3O_5^+$[M+H]$^+$ 392.12410. Found: 392.12397.

Ethyl 1-((3-(4-methoxyphenyl)isoxazol-5-yl)methyl)-1H-indole-2-carboxylate **(8h)**

Yield 31.8%; yellow solid; m.p: 144 – 146°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.27 (t, J = 7.1 Hz, CH_2CH_3), 3.84 (s, Ar-OCH_3), 4.29 (q, J = 7.1 Hz, OCH_2CH$_3$), 5.99 (s, NCH_2C), 6.76 (s, H-4'), 7.07 – 7.38 (m, H-6, H-3'', H-5''), 7.65 -7.74 (m, H-3, H-6), 7.82 (d, J = 2.1 Hz, H-2'', H-6''). ^{13}C NMR (75 MHz, DMSO-d$_6$): δ = 14.6 (CH_2CH_3), 40.7 (NCH_2C), 56.8 (OCH_3), 61.2 (OCH_2CH$_3$), 100.7 (C-4'), 111.5 (C-3), 111.7 (C-7), 113.7 (C-3'', C-5''), 121.7 (C-5), 122.0 (C-4), 122.2 (C-1''), 123.1 (C-6), 126.1 (C-2'', C-6''), 127.3 (C-3a), 127.5 (C-2), 139.4 (C-7a), 156.4 (C-5'), 161.1 (Ar-COO), 161.6 (C-4''), 170.2 (C-3'). HRMS (ESI) m/z: Calcd. for $C_{22}H_{20}N_2O_4^+$[M+H]$^+$ 377.14958. Found: 377.14969.

2.2.7 Grwoth and Maintainance of Microorganisms and Cancer Cell Lines

The synthesized compounds were tested against Gram-positive strains namely; Methicillin-sensitive *Staphylococcus aureus* NCTC 10788 (MSSA), methicillin-resistant *Staphylococcus aureus* ATCC 33591 (MRSA), two standard Gram-negative bacterial strains namely; *Escherichia coli* NCTC 12923, *Pseudomonas aeruginosa* NCTC 12924 along with the two fungi *Candida albicans* (NCPF 3179) and *Aspergillus brasiliences* (NCPF 2275). The microorganisms were inoculated into Trypticase soy broth and Sabaroud dextrose broth for bacteria and fungi respectively and the pH of the medium was adjusted to 7.3 with sterile phosphate buffered saline. The cultures were subsequently incubated at 37°C for 24-48 hours except for Aspergillus which was incubated at 25°C for 5-7 days. The optical density (OD) of the bacteria from mid-log phase of growth was measured at 600 nm and to obtain an optical density of 0.4 (corresponding to 1.5×10^8 colony forming units/mL).

MCF-7 (human breast adenocarcinoma), Hep2 (human epiglottis cancer), and Hela (cervical cancer cells) cell cultures were kindly provided by Jordan Company for Antibody Production (MONOJO, Amman, Jordan). The cell lines were cultured in growth medium (RPMI-1640medium, pH7.4), supplemented with 15% fetal bovine serum (FBS) and antibiotics [(penicillin (100 units/mL) and streptomycin (100 ug/mL)]. The cell lines were maintained at 37°C in a 5% CO_2 atmosphere with 95% humidity [33].

2.2.8 Antimicrobial Susceptibility Testing

The stock suspensions of microorganisms were prepared to the standard McFarlands (0.5) and subsequently uniformly spread on a solid growth medium in a Petri dish. Sterile paper disks (6 mm in diameter; Becton, Dickinson & Co.) were placed on the agar plates and impregnated with 30 uL solution of each compound. Plates were incubated for the recommended time periods (24–48 h and up to 7 days for Aspergillus) under appropriate cultivation conditions. Antimicrobial activity was determined if the tested compound produced an inhibition zone around the impregnated disk. Disks impregnated with sterile DMSO served as negative controls and disks with standard antibiotics (vancomycin, ciprofloxacin, and clarithromycin (Oxoid, UK)) served as positive controls. Measurements at each concentration were performed in triplicates [36]. For MIC determination, 200 µL of diluted microbial suspension was added, (0.2-500 µg/50 µL) of the synthesized compounds and standard antibiotics (Cephtriaxone, Gentamycin and Levofloxacin) in 20% H_2O/DMSO were added to wells of ELISA plates, and subsequently incubated at conditions described above. The effect of the tested compounds on the growth of microorganisms was monitored by measuring the optical density at 600 nm using an ELISA reader. The MIC was defined as the lowest concentration of the tested compounds allowing no visible growth. All measurements of MIC values were repeated in triplicate.

Antimicrobial interactions between the tested compounds conventional antimicrobial agents like cefzolidin and amoxicillin against MRSA were evaluated by the standard checkerboard titration method [34]. The bacterial suspensions, growth media, and culture conditions were the same as those described for the MIC determination mentioned above. Experiments were performed in triplicate. The fractional inhibitory concentrations (FICs) were calculated as follows:

FIC = (MIC of drug A in combination/MIC of drug A alone) + (MIC of drug B in combination/MIC of drug B alone).

The FIC indices were interpreted as follows: ≤0.5, synergy; 0.5–1, additive; 1–4.0, indifference; >4.0, antagonism [37].

2.2.9 Cell Cytotoxicity for Anticancer Activity Testing

Microculture tetrazolium (MTT) assay was used to evaluate cell vitality. Briefly, monolayers of each cell line were trypsinized and the cells were seeded in 96-well plates at the density of 5 x10^5 cell/well (100 µL/well) in a culture medium and incubated for 24 h at 37°C, with 5% CO_2 in a humidified atmosphere. The medium was subsequently removed and fresh growth medium containing different concentrations of tested compounds (concentration range

of 5-500 µg/mL) were separately added. Control cells were supplemented only with a medium and colchicine was used as a positive control. 20 µL of 5 mg / mL MTT (pH 7.4) in 180 µL media was added per well and incubated for another 4 h. The plates were then centrifuged and supernatants were removed to all the addition of 100 µL of 1:1 ethanol: DMSO. The absorbance was then measured by an ELISA reader Bio-Rad, USA) at a wavelength of 570 nm. The values are presented as means of duplicate analyses. The activity of the tested compounds on the proliferation of cancer cells was expressed as the % of cytotoxicity [36].

3 Conclusion

In summary, sixteen new hybrid compounds were synthesized featuring the indole and the isoxazole moieties. All new compounds were identified and tested against three bacterial strains and three cancer cell lines. It was found through this research work that these newly-synthesized compounds did not show any promising activities. Single X-ray crystal structure was determined for a representative compound of these new hybrid systems.

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Conflict of interest: Authors declare no conflict of interest.

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