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Synthesis, Anticancer and Antimicrobial Activities of New 6-(1*H*-1,2,3-Triazol-4-yl)imidazo[2,1-*b*]thiazoles

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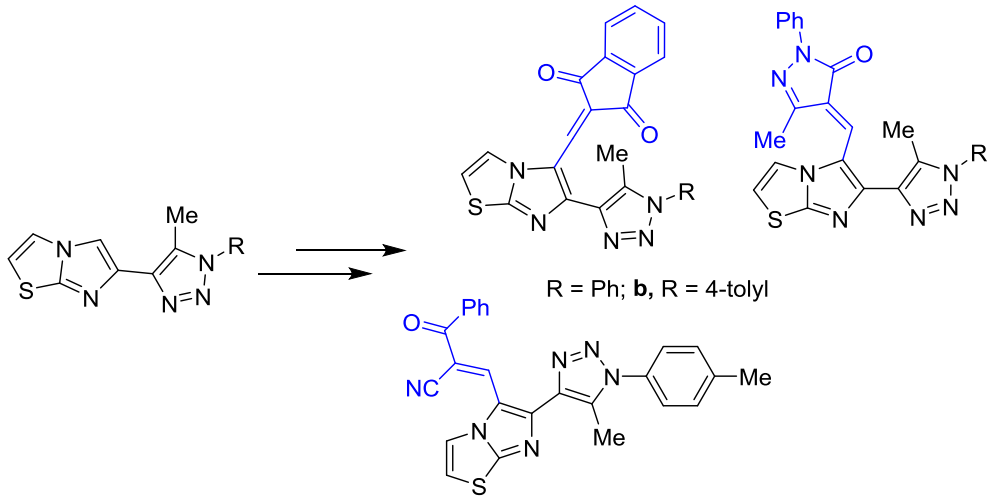
Abstract

Novel 6-(1*H*-1,2,3-triazol-4-yl)-imidazo[2,1-b]thiazoles **8-10** were synthesized in good yields by Claisen–Schmidt reaction of the active methylene compounds such as pyrazol-5-one **5**, 1,3-indandione **6** and benzoylaceteonitrile **7**, with imidazo[2,1-*b*]thiazol-5-aldehydes **4** in basic medium. Anti-tumor activities in vitro against HepG-2, PC-3 and HCT-116 human carcinoma cell lines were tested for new products. Both compounds **9a** and **9b** showed antitumor activity comparable to that of positive control (Doxorubicin®). Also, the antimicrobial activities of novel compounds were evaluated. Amongst the tested compounds **9a**, **9b**, and **10** exhibited excellent antimicrobial activity.



Keywords: 1,2,3-Triazole; Imidazo[2,1-b]thiazole; Anticancer; Antimicrobial activity, Single crystal x-ray.

1. Introduction

1,2,3-Triazoles have a great utility in medicinal chemistry because they show a wide range of biological profiles that includes anti-cancer activities [1,2], antimicrobial [2], and anti-inflammatory [3]. There are many drugs containing 1,2,3-triazole moiety on the market such as carboxyamidotriazole (CAI, anticancer) [4], tert-butyldimethylsilylspiroaminooxathioledioxide (TSAO, inhibitors of HIV-1 reverse transcriptase dimerization) [5], Tazobactum (β-lactam antibiotic) (Fig.1). Also, imidazo[2,1-*b*]thiazoles have different pharmacological properties such as antimicrobial [6-12], anti-inflammatory [13], and antitumor activities against some human cancer cells [14-20]. For all of these above advantages and continued to our work

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[21-23], we report herein the synthesis of some new 6-(1*H*-1,2,3-triazol-4-yl)-imidazo[2,1-b]thiazoles to explore their anticancer and antimicrobial activity.

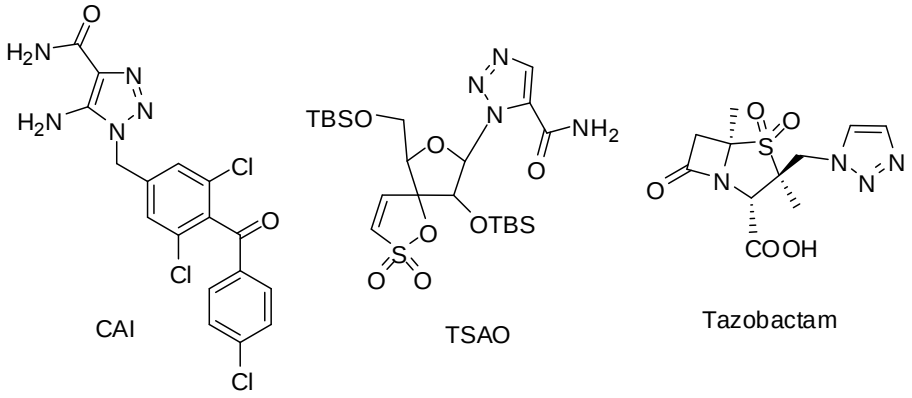


Fig. 1: Potential pharmaceuticals based on 1,2,3-triazoles.

2. Experimental

2.1. General Techniques

All melting points were taken on Electrothermal IA 9000 series digital melting point apparatus. Elemental analytical data were carried from the micro analytical unit, Cairo University, Giza, Egypt. The IR spectra were recorded in potassium bromide disks on a JASCO FT/IR-6100. ^{1}H-NMR spectra were run on JOEL-ECA 500MHz in deuterated dimethyl sulphoxide (DMSO-d6). Chemical shifts values (δ) are given in parts per million (ppm). The mass spectra were performed using mass Varian MAT CH-5 spectrometer at 70eV. 4-Bromoacetyl-5-methyl-1,2,3-triazoles 2, [24] 6-(5-methyl-1,2,3-triazol-4-yl)imidazo[2,1-b]thiazoles **3a,b** and 6-(5-methyl-1,2,3-triazol-4-yl)imidazo[2,1-*b*]thiazole-5-carbaldehydes **4a,b** [25] were prepared according to literature.

2.1.1. General procedures for synthesis of compounds 8-10

A mixture of appropriate 6-(5-Methyl-1,2,3-triazol-4-yl)imidazo[2,1-*b*]thiazole-5-carbaldehyde **4** (1 mmol) and appropriate active methyelene competent **5** , **6** or **7** (1 mmol) in ethanol (25mL) and few drops of piperidine was refluxed for 4-6 h (TLC). The resulting solid was collected by filtration.

2.1.1.1. 3-Methyl-4-((6-(5-methyl-1-phenyl-1*H*-1,2,3-triazol-4-yl)imidazo[2,1-*b*]thiazol-5-yl)methylene)-1-phenyl-1*H*-pyrazol-5(4*H*)-one (8a)

Yield 73 %; m.p. 272-273 °C; IR (KBr) νmax/cm^{-1} 1665 (C=O); ^{1}H NMR (DMSO-d$_6$) δ 2.34, 2.44 (2s, 6H, 2CH$_3$), 7.45-7.56 (m, 10H, Ar-H), 7.85(d, 1H, thiazole-H, J = 8 Hz), 7.89 (d, 1H, thiazole-H, J = 8 Hz), 8.22 (s, 1H, olefinic-H); MS m/z (%): 465 (M+, 78), 91(100); Anal. Calcd for $C_{25}H_{19}N_7OS$ (465.53): C, 64.50; H, 4.11; N, 21.06 %. Found: C, 65.68; H, 4.32; N, 21.19.

2.1.1.2. 3-Methyl-4-((6-(5-methyl-1-*p*-tolyl-1*H*-1,2,3-triazol-4-yl)imidazo[2,1-*b*]thiazol-5-yl)methylene)-1-phenyl-1*H*-pyrazol-5(4*H*)-one (8b)

Yield 75 %; m.p. 284-285 °C; IR (KBr) νmax/cm^{-1} 1658 (C=O); ^{1}H NMR (DMSO-d$_6$) δ 2.34, 2.44, 2.49 (3s, 9H, 3CH$_3$), 7.45-7.56 (m, 9 H, Ar-H), 7.90 (d, 1H, thiazole-H, J = 8 Hz), 7.91 (d, 1H, thiazole-H, J = 8 Hz), 8.24 (s, 1H, olefinic-H); MS m/z (%): 479 (M+, 51), 91(100); Anal. Calcd for $C_{26}H_{21}N_7OS$ (479.56): C, 65.12; H, 4.41; N, 20.45 %. Found: C, 65.23; H, 4.62; N, 20.57.

2.1.1.3. 2-((6-(5-Methyl-1-phenyl-1*H*-1,2,3-triazol-4-yl)imidazo[2,1-*b*]thiazol-5-yl)methylene)-1*H*-indene-1,3(2*H*)-dione (9a)

Yield 56 %; m.p. 289-290 °C; IR (KBr) νmax/cm^{-1} 1710 (2 C=O); ^{1}H NMR (DMSO-d$_6$) δ: 2.35 (s, 3H, CH$_3$), 7.45-7.87 (m, 9 H, Ar-H), 7.89 (d, 1H, thiazole-H, J = 8 Hz), 7.90 (d, 1H, thiazole-H, J = 8 Hz), 8.64 (s, 1H,olefinic-H); MS m/z (%): 437 (M+, 98), 91(100); Anal. Calcd for $C_{24}H_{15}N_5O_2S$ (437.47): C, 65.89; H, 3.46; N, 16.01 %. Found: C, 65.94; H, 3.65; N, 16.21.

2.1.1.4. 2-((6-(5-Methyl-1-*p*-tolyl-1*H*-1,2,3-triazol-4-yl)imidazo[2,1-*b*]thiazol-5-yl)methylene)-1*H*-indene-1,3(2*H*)-dione (9b)

Yield 61 %; m.p. 285-286 °C; IR (KBr) νmax/cm^{-1} 1714 (2 C=O); ^{1}H NMR (DMSO-d_6) δ: 2.35, 2.42 (2s, 6H, 2CH_3), 7.45-7.87 (m, 8 H, Ar-H), 7.89 (d, 1H, thiazole-H, J = 8 Hz), 7.90 (d, 1H, thiazole-H, J = 8 Hz), 8.66 (s, 1H, olefinic-H); MS m/z (%): 451 (M+, 98), 91(100); Anal. Calcd for $C_{25}H_{17}N_5O_2S$ (451.50): C, 66.50; H, 3.80; N, 15.51 %. Found: C, 66.73; H, 4.89; N, 15.67.

2.1.1.5. 2-Benzoyl-3-(6-(5-methyl-1-*p*-tolyl-1*H*-1,2,3-triazol-4-yl)imidazo[2,1-*b*]thiazol-5-yl)acrylonitrile (10)

Yield 48 %; m.p. 300 °C; IR (KBr) νmax/cm^{-1} 1698 (C=O), 2196 (CN); ^{1}H NMR (DMSO-d_6) δ 2.39, 2.44 (2s, 6H, 2CH_3), 7.39-7.63 (m, 9 H, Ar-H), 7.96 (d, 1H, thiazole-H, J = 8 Hz), 7.98 (d, 1H, thiazole-H, J = 8 Hz), 9.71 (s, 1H, olefinic-H); MS m/z (%): 450 (M+, 33), 57(100); Anal. Calcd for $C_{25}H_{18}N_6OS$ (450.52): C, 66.65; H, 4.03; N, 18.65 %. Found: C, 66.77; H, 4.34; N, 18.86.

2.2. X-ray crystallography of compound 9b

A single crystal of compound **9b** was obtained by slow evaporation at room temperature, from dimethylformamide (DMF). The crystal structure was solved and refined using MaXus (Bruker Nonius, Deflt and MacScience, Japan) [26] Mo Kα radiation (λ = 0.71073 Å) and a graphite monochromator were used for data collection. The chemical formula and ring labeling system is shown in Fig. 1. Crystal data for compound **9b**: $C_{24}H_{15}N_5O_2S$, Mr, 437.481; system, orthorhombic; Space group, $P^{-}1$; unit cell dimensions, a, 13.0923 (8)Å; b, 7.1822 (6)Å; c, 21.467 (2)Å; α, 90.00°; β, 96.298 (5)°; γ, 90.00°; V, 2006.4 (3)Å3; Z, 4; Dx, 1.448 Mg m-3; θ range for data collection, 27.45 °; μ (Mo- Kα), 0.20 mm-1; T = 298 K; independent reflections, 5701; measured reflections, 4653; observed reflections, 429; Rint, 0.222; R(all), 0.419; R(gt), 0.020; wR(ref), 0.042; wR(all), 0.210; wR(gt), 0.042; S(ref), 1.388; S(all), 1.785; S(gt), 1.388; Δ/σmax, 0.018, Δρmax, 1.66eÅ3; Δρmin-1.62eÅ3.

Crystallographic data for the structures 9b have been deposited with the Cambridge Crystallographic Data Center (CCDC) under the number 1064048. Copies of the data can be obtained, free of charge, on application to CCDC 12 Union Road, Cambridge CB2 1EZ, UK [Fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk or at www.ccdc.cam.ac.uk.

2.3. Biological activity

2.3.1. In-vitro anticancer activity

Cell culture of HepG-2 (human liver carcinoma), PC-3 (human prostate adenocarcinoma) and HCT116 (human colorectal carcinoma) cell lines were purchased from the American Type Culture Collection (Rockville, MD) and maintained in RPMI-1640 medium which was supplemented with 10% heat-inactivated FBS (fetal bovine serum), 100U/ml penicillin and 100U/ml streptomycin. The cells were grown at 37°C in a humidified atmosphere of 5% CO_2.

2.2.2. MTT cytotoxicity assay

The anticancer activity against HepG-2, PC-3 and HCT-116 human cancer cell lines was estimated using the 3-[4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, which is based on the cleavage of the tetrazolium salt by mitochondrial dehydrogenases in viable cells [27-29]. Cells were dispensed in a 96 well sterile microplate (5 x 104 cells/well), and incubated at 37^{o}C with series of different concentrations, in DMSO, of each tested compound or Doxorubicin® (positive control) for 48 h in a serum free medium prior to the MTT assay. After incubation, media were carefully removed, 40 μL of MTT (2.5 mg/mL) were added to each well and then incubated for an additional 4 h. The purple formazan dye crystals were solubilized by the addition of 200 μL of DMSO. The absorbance was measured at 590 nm using a SpectraMax® Paradigm® multi-Mode microplate reader. The relative cell viability was expressed as the mean percentage of viable cells compared to the untreated control cells.

2.3.3. Statistical analysis

All experiments were conducted in triplicate and repeated in three different days. All the values were represented as mean ± SD. IC50s were determined by probit analysis using SPSS software program (SPSS Inc., Chicago, IL).

2.4. Antimicrobial activity

Chemical compounds were individually tested against a panel of gram positive and gram-negative bacterial pathogens, yeast and fungi. Antimicrobial tests were carried out by the agar well diffusion method [30] using 100 μL of suspension containing 1x10^8 CFU/mL of pathological tested bacteria and 1x10^6 CFU/ml of yeast spread on nutrient agar (NA) and Sabourand dextrose agar (SDA) respectively. After the media had cooled and solidified, wells (10 mm in diameter) were made in the solidified agar and loaded with 100 μL of tested compound solution

prepared by dissolving 200 mg of the chemical compound in 1 ml of dimethyl sulfoxide (DMSO). The inculcated plates were then incubated for 24 h at 37 °C for bacteria and 48h at 28°C for fungi. Negative controls were prepared using DMSO employed for dissolving the tested compound. (Ciprofloxacin (50 mg/ml and Ketoconazole (50 mg/ml) were used as standard for antibacterial and antifungal activity respectively. After incubation time, antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms and compared with that of the standard. The observed zone of inhibition is presented in Table 1. Antimicrobial activities were expressed as inhibition diameter zones in millimeters (mm). The experiment was carried out in triplicate and the average zone of inhibition was calculated.

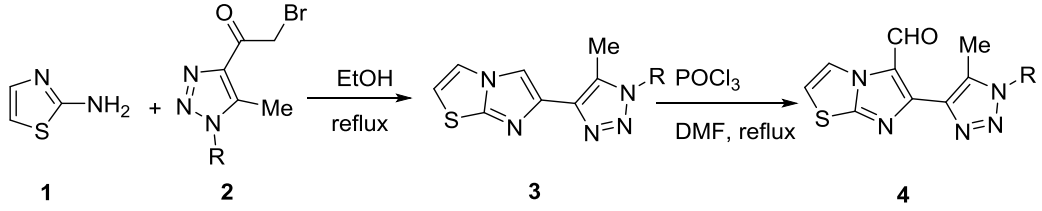
2.4.1. Minimal inhibitory concentration (MIC) measurement

The bacteriostatic activity of the active compounds (having inhibition zones (IZ) ≥ 16 mm) was then evaluated using the two-fold serial dilution technique [31]. Two-fold serial dilutions of the tested compounds solutions were prepared using the proper nutrient broth. The final concentration of the solutions was 200, 100, 50, 25 µg/ml. Each 5 ml received 0.1 ml of the appropriate inoculum and incubated at 37 °C for 24hours and 48 h at 28°C for fungi. The lowest concentration showing no growth was taken as the minimum inhibitory concentration (MIC).

3. Results and Discussion

3.1. Chemistry

Our approach towards the policy and progress of new compounds involves the use of imidazo[2,1-b]thiazol-5-aldehydes **4**. The reaction of 2-aminothiazole **1** with 2-bromo-1-(1*H*-1,2,3-triazol-4-yl)ethan-1-ones **2** at reflux temperature afforded imidazo[2,1-*b*]thiazoles **3**. Application of Vilsmeier-Haack reaction on compound **3** afforded the desired compound **4** (Scheme 1).



2-4; a, R = Ph; **b,** R = 4-tolyl

Scheme 1: Synthesis of 6-(5-methyl-1-aryl-1H-1,2,3-triazol-4-yl)imidazo[2,1-b]thiazole-5-carbaldehydes (**4**)

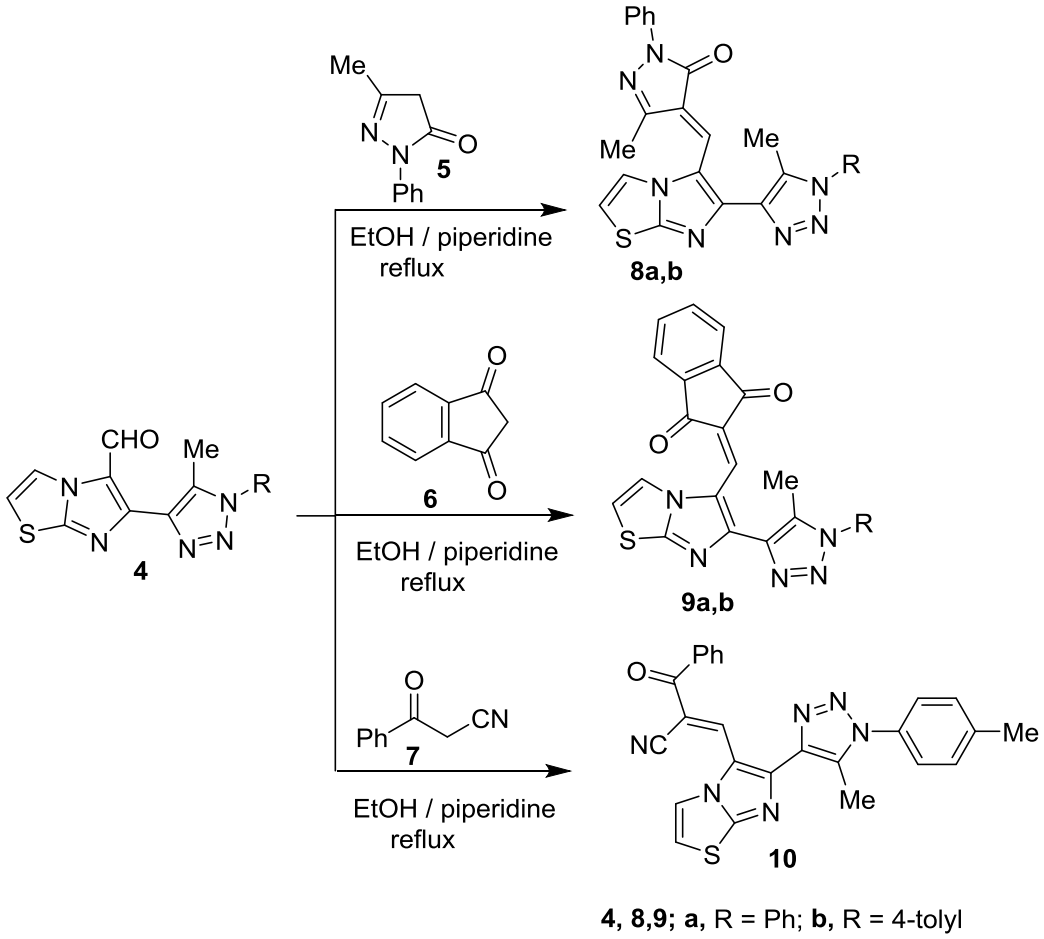
Claisen–Schmidt condensation reaction of the active methylene compounds, namely, 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one **5**, 1,3-indandione **6** or benzoylaceteonitrile **7**, with imidazo[2,1-*b*]thiazol-5-aldehydes **4** in absolute ethanol containing catalytic amount of piperidine afforded various imidazothiazoles **8a**,**b**; **9a,b**; and **10**; respectively as shown in Scheme 2.

The IR spectrum of compound **10** showed the presence of cyano group at wave number 2196 cm^{-1}. Also, the ^{1}H NMR spectra of **9-10** showed the absence of aldehyde group proton. The chemical structure of compound **9b** was confirmed by the X-ray crystallography (crystallized from DMF) (Fig. 1).

3.2. Biological Evaluation

3.2.1. Anti-tumor activity

The anti-tumor activities against HepG-2, PC-3 and HCT-116 human carcinoma cell lines using MTT assay were tested for new products. The IC_{50} values are shown in Table 1, we can note that both compounds **9a** and **9b** showed anticancer activities comparable to that of positive control (Doxorubicin®) against HCT-116 carcinoma cells. Compounds **10** and **8b** showed slightly lower anticancer activities than those of the positive control and compound **8a** showed moderate activity against HCT-116. In addition, compound **9b** showed better anticancer activity and compound **8b** showed slightly lower activity compared to positive control against PC-3 cancer type; however, compounds **9a** and **10** showed moderate activities and only compound **8a** did not show any activity against PC-3 caner type. Also, all new synthetic compounds showed weak antitumor activity against HepG-2.



Scheme 2: Synthesis of compounds **8**, **9**, and **10.**

Table 1: The IC50 values of the anticancer activities for compounds **8a**, **8b**, **9a**, **9b**, and **10** against three cancer types, using MTT assay.

Compounds	HCT-116	PC-3	HepG2
	IC_{50} (µg/mL)		
8a	81.14	> 1000	172.68
8b	58.73	70.96	141.09
9a	55.34	73.81	152.51
9b	54.77	66.64	143.85
10	60.49	85.39	199.97

3.2.2. Antimicrobial activity

The antimicrobial activity of all new compounds was studied, at 100 µg/mL concentration, against three Gram positive bacteria and three Gram negative bacteria and two yeast. Standard antibacterial and antifungal agents Ciprofloxacin and Ketoconazole are used. Antibacterial and antifungal activities are displayed in Table 2. All compounds have superior significant. Compounds **9a** and **9b** offered the highest activity against all the tested bacteria and fungi with inhibition ranges ranging from 29 to 33 mm.

Minimum inhibitory concentration (MIC)

The results are shown in Table 3 displayed the results MIC for the synthesized compounds. Compounds **9a** and **9b** exhibited the highest MIC (25 µg/ml) against all tested microorganisms while compounds **8a** and **8b** showed lower MIC (200 µg/ml) against Pseudomonas Aeroginosa ATCC27953 and E. coli ATCC25922.

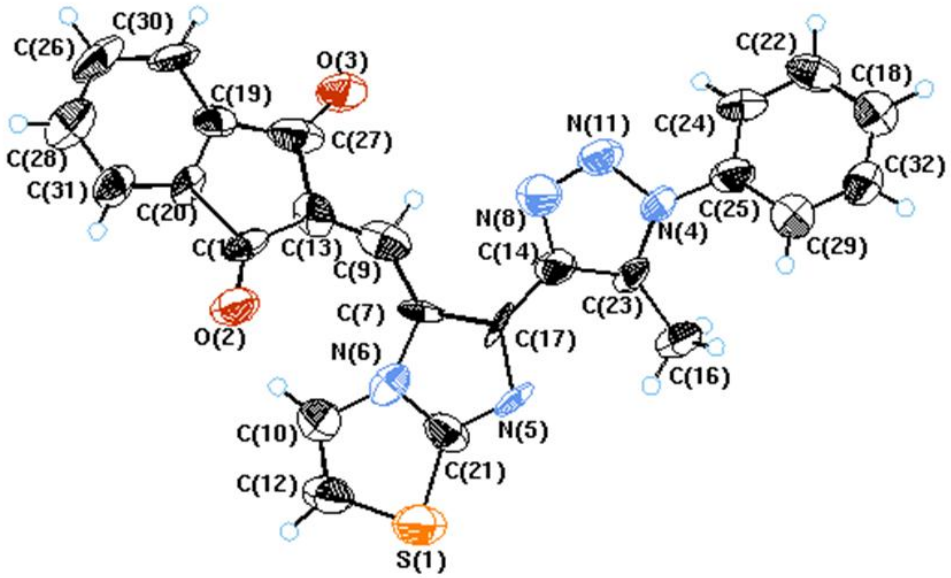


Fig. 1: X-ray structure of 9b

Table 2: Antimicrobial activity for new synthetic compounds (mm)

	Gram positive bacteria			Gram negative bacteria			Yeast	
Compounds	*Staphelococcus aureus* ATCC 29213	*B. subtilis ATCC6633*	*B. megaterium ATCC* 9885	*Klebseilla peneumoniae* ATCC13883	*Pseudomonas. Aeroginosa* ATCC27953	*E. coli ATCC 25922*	*Saccharomyces cervesia*	*Candida Albicans* NRRL Y-477
8a	26	22	28	20	21	19	24	20
8b	20	22	20	26	20	22	23	22
9a	30	31	33	29	33	30	31	31
9b	33	32	33	30	33	33	31	32
10	28	28	27	29	22	27	29	26
Ciprofloxacin	20	22	24	25	24	23	N.A.	N.A.
Ketoconazole	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	23	22

The experiment was carried out in triplicate and the average zone of inhibition was calculated

Table 3: Minimum inhibitory concentration (µg/ml)

Compounds	Gram positive bacteria			Gram negative bacteria			Yeast	
	Staphelococcus aureus ATCC 29213	*B. subtilis ATCC6633*	*B. megaterium ATCC* 9885	*Klebseilla peneumoniae* ATCC13883	*Pseudomonas. Aeroginosa* ATCC27953	*E. coli ATCC 25922*	*Saccharomyces cervesia*	*Candida Albicans* NRRL Y-477
8a	100	200	100	200	200	200	100	200
8b	100	100	200	50	200	200	100	100
9a	25	25	25	25	25	25	25	25
9b	25	25	25	25	25	25	25	25
10	50	50	50	50	50	50	50	50
Ciprofloxacin	25	25	25	25	25	25	N.A.	N.A.
Ketoconazole	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	25	25

The experiment was carried out in triplicate and the average zone of inhibition was calculated

Conclusions

Claisen–Schmidt reaction was used for the synthesis of new imidazo[2,1-*b*]thiazoles compounds **8-10**. These new compounds showed anti-tumor activities comparable to that of positive control (Doxorubicin®). Also, some of these compounds showed good antimicrobial activities.

Conflicts of interest

The authors declare no conflict of interest

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