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A facile design and efficient synthesis of schiff's bases of tetrazolo [1,5-*a*] quinoxalines as potential anti-inflammatory and anti-microbial agents

Umarani Natarajan*, Ilango Kaliappan, Narendra Kumar Singh

Department of Pharmaceutical Chemistry, SRM College of Pharmacy, SRM University, Kattankulathur, India

Abstract

A novel synthetic methodology of schiff's bases incorporating tetrazolo quinoxalines is described. O-Phenylene diamine on condensation with oxalic acid using Phillip's procedure yielded the corresponding quinoxaline-2,3-dione **1**. The latter on chlorination afforded 2,3-dichloroquinoxaline **2**. This on hydrazinolysis gave the known 3-chloro-2-hydrazino quinoxaline **3**, which further undergoes cyclisation with sodium azide to obtain 2-hydrazino tetrazolo [1,5-*a*]quinoxaline **4**. Finally this synthesized scaffold made to react with different aromatic aldehydes furnished the formation of 1-substituted hydrazino tetrazolo [1, 5-*a*] quinoxalines **5a-j**. The structures of all these title compounds were confirmed by their IR, ^{1}HNMR, and Mass spectral analysis. All the newly synthesized heterocycles have been screened for their *in vitro* antimicrobial and anti-inflammatory activities. Few of them exhibited promising activity. The ambient conditions, excellent product yields and easy work up procedures make this synthetic strategy a better protocol for the synthesis of newer schiff's derivatives.

Keywords: Tetrazolo quinoxalines, scaffold, spectral studies, synthetic strategy.

Introduction

The exploration of privileged structures in drug discovery has gained significant popularity in medicinal chemistry over the past years. Heterocycles play an important role in the design and discovery of new pharmacologically active compounds. Recently quinoxalines and related heterocycles were introduced as prospective potential chemotherapeutic drug candidates

possessing manifold biological activities [1-8]. Besides a wide spectrum of pharmacological activities, quinoxaline derivatives have been reported to possess anti-microbial [9] and anti-inflammatory [10] activities. Moreover fusion of tetrazole, which is considered as planar acidic heterocyclic analogue of carboxylic function [11,12], has the ability to increase potency [13,14] and improve bioavailability[15]. Similarly schiff's bases apart from other biological activities have been reported to exhibit antimicrobial effect [16,17]. In the light of above findings and coupled with our interests of the chemistry of bridge head nitrogen heterocyclic systems gave us the idea for the synthesis of novel condensed heterocyclic compounds encompassing bioactive molecules tetrazoles and quinoxalines with the aim to explore their potent biological activity for the first time. Lately, new biological effects as well as a number of biological targets for these compounds have been discovered, paving the way for drug like substances design on their basis.

Results and Discussion

Chemistry

The target compounds were synthesized according to the representative scheme 1. The required starting material, quinoxaline-2,3-dione **1** was prepared in good yield by condensation of o-Phenylene diamine with oxalic acid in 4N HCl using Phillip's procedure [18]. The subsequent chlorination reaction of **1** with phosphorous oxychloride at room temperature gave the known 2,3-dichloro quinoxaline **2**, which on hydrazinolysis afforded the corresponding 3-chloro-2-hydrazino quinoxaline **3**. The compound **3** undergoes cyclisation with sodium azide in ethanol to obtain 2-hydrazino tetrazolo [1,5-*a*] quinoxaline **4**. The scaffold synthesized was finally treated with different substituted aromatic aldehydes resulted in the formation of schiff's bases of title compounds **5a-j**. The yields of all the synthesized compounds were found to be in the range of 62-88%.

IR Spectral Studies

Assignments of selected characteristic IR band positions provide significant indication for the formation of 1-substituted benzylidene hydrazino tetrazolo [1,5-*a*]quinoxalines. The infrared spectroscopic investigation of all the compounds **5a-j** showed sharp peak at 3340-3420 cm^{-1} due to the presence of NH stretching of NH_2, an intense band of 1618 cm^{-1} to 1632 cm^{-1} attributed to the appearance of -N=N stretching, sharp bands at 1523cm^{-1} and 1561cm^{-1} evidenced the appearance of -C-N and -C=N stretching. The asymmetric vibrations of the aromatic nitro group in the compounds **5e** and **5f** exhibited a peak of medium intensity at 1565cm^{-1}.

NMR Spectral Studies

Further evidence for the formation of target compounds were obtained from the ^{1}HNMR spectra, which proved to be a diagnostic tool for the positional elucidation of the proton. Assignments of the signals are based on the chemical shift and intensity pattern. The formation of schiff's bases was evidenced by the disappearance of NH_2 protons and appearance of azomethine (-N=CH) proton peak at δ ppm 8.42. The multiplet signals at δ ppm 7.23-8.45 are the characteristics of aromatic ring protons. A sharp signal appears at δ ppm 3.92 is the characteristics of the protons of -OCH_3. A broad signal at δ ppm 11.42, due to the characteristics of -NH proton was accordance with all the proposed structures.

Mass spectral analysis

Final proof for the structures were obtained by recording its mass spectrum, which exhibited a molecular ion peak at m/z 305, 334, 323, 332, and 379 respectively corresponding to its molecular weight. The mass spectral data of all the titled compounds were found to be in correlation with the expected structure.

***In vitro* Antimicrobial activity**

A series of schiff's bases of tetrazolo quinoxalines were prepared and tested for their *in vitro* antimicrobial activity against six strains of microbes, which are *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Aspergillus niger* and *Candida albicans*. It was observed that the synthesized compounds having electron withdrawing groups showed very good antimicrobial properties. The target compounds **5d** and **5f** were excellently equipotent against the microbial strains, **5g** and **5h** showed optimum equipotent activity, **5j** was moderately active and **5i** was mild active against the strains. Thus the substitution of electron withdrawing groups in C-2 and C-4 of the phenyl ring seems to be of great significance for antimicrobial efficacy.

***In vitro* Anti-inflammatory activity**

The synthesized target compounds exhibited membrane stabilization effect by inhibiting hypotonicity-induced lyses of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane [19] and its stabilization implies that the title compounds may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release [20]. Some of the NSAIDS are known to possess membrane stabilization properties which may contribute to the potency of their anti inflammatory effect. Though the exact mechanism of the membrane stabilization is not known yet, hypotonicity - induced haemolysis may arise from shrinkage of the cells due to osmotic loss of intracellular electrolyte and fluid components. The test compounds may inhibit the processes, which may stimulate or enhance the efflux of these intracellular components [21].Out of the entire synthesized target compounds, compound **5i** showed the maximum anti-inflammatory activity closely followed by the compound **5j**, where as the rest of the remaining compounds **5a-h** showed moderate anti - inflammatory activity. The reason for the increased activity of **5i** and **5j** was attributed to the presence of hydrophobic and electron-donating nature of the tri methyl amino moiety in **5i** and methoxy group in **5j** at the phenyl ring.

Thus it was observed that for the very reason **5i** and **5j** showed maximum anti- inflammatory and they have minimal antimicrobial activities. Similarly the rest of the compounds in series showed moderate anti-inflammatory but excellent antimicrobial properties.

Conclusion

In conclusion, a new class of tetrazolo quinoxalines heterocycles was synthesized and the results of antimicrobial and anti-inflammatory data revealed that the compounds possess significant *in vitro* activity. Therefore, this study would be a fruitful matrix for the development of novel class of schiff's bases of tetrazolo quinoxalines as interesting lead molecules for further synthetic and

biological evaluation. It is convincing that this class of compounds certainly holds great promise towards the pursuit to discover novel classes of antimicrobial and anti - inflammatory agents. Further studies to acquire more information concerning structural activity relationships are in progress.

Materials and Methods

All chemicals used were of analytical grade and purchased from SD Fine. Chemicals (Mumbai, India). The melting point of the compounds was determined on a Veego digital melting point apparatus (Model VMP-D) and the values are uncorrected. The purity of the compounds and progress of reaction was monitored by thin layer chromatography (TLC) using precoated silica gel G aluminium plates (0.5mm thickness, E-Merck) and spots were visualized using UV radiation/iodine vapour. Infra red absorption spectra were scanned in the 4000-400cm^{-1} ranges using KBr discs on FTIR Shimadzu Spectrometer. ^{1}HNMR spectra were scanned at 300 MHZ on a Bruker Avance II Model Spectrophotometer using TMS as internal reference standard and chemical shifts (δ) are quoted in ppm. Mass spectra were acquired on Shimadzu GC-MS QP-2010. The physicochemical properties of title compounds were summarized in Table 1.

Table 1. The characterization data of title compounds 5(a-j)

Compound	Ar	Mol. For	R_f value	Percentage yield (%)
5a	2-OH- (C_6H_4)	$C_{15}H_{11}N_7O$	0.66	66
5b	3-OH- (C_6H_4)	$C_{15}H_{11}N_7O$	0.67	72
5c	4-OH- (C_6H_4)	$C_{15}H_{11}N_7O$	0.65	62
5d	2-NO_2- (C_6H_4)	$C_{15}H_{10}N_8O_2$	0.72	85
5e	3-NO_2- (C_6H_4)	$C_{15}H_{10}N_8O_2$	0.72	88
5f	4-NO_2- (C_6H_4)	$C_{15}H_{10}N_8O_2$	0.77	78
5g	2-Cl- (C_6H_4)	$C_{15}H_{10}O_7Cl$	0.62	83
5h	4-Cl- (C_6H_4)	$C_{15}H_{10}O_7Cl$	0.64	85
5i	2-N $(CH_3)_2C_6H_4$	$C_{17}H_{16}N_8$	0.73	87
5j	3, 4, 5- $(OCH_3)_3C_6H_2$	$C_{18}H_{17}N_7O_3$	0.69	75

General procedures

Synthesis of quinoxalin-2, 3-dione (1)
To a mixture of o-Phenylene diamine (27.9g, 0.25 mole) and oxalic acid (32.5g, 0.36 mole) 4N HCl (150ml) was added and refluxed in an oil bath for 1 hr and cooled. The crude solid that separated out was filtered, washed and recrystallised from ethanol. The yield of the product was 86%. mp.>300^{o}C, MS: (M^{+}) 162.

Synthesis of 2, 3-dichloroquinoxaline (2)
An equimolar quantity of quinoxalin-2,3-dione **1** (16.01g, 0.10 mole) was treated with phosphorous oxychloride (15.33g, 0.10 mole) at room temperature and allowed to stand for 1 hr. The resultant product obtained was recrystallised from ethanol. The yield of the product was 82%. mp. 264-268^{o}C, (M^{+}) 197.

Synthesis of 3-chloro-2-hydrazino quinoxaline (3)
To a mixture of 2,3-dichloroquinoxaline **2** (2.98g, 0.015 mole) and hydrazine hydrate (1g, 0.02 mole), 50 ml of ethanol was added and refluxed for 3 hr. The solid mass obtained was filtered and recrystallised from ethanol. The yield of the product was 84%. mp. 187-190^{o}C, Mass (m/z): 194.

Synthesis of 2-hydrazino tetrazolo [1,5-*a*] quinoxaline (4)
To an ethanolic solution of 3-chloro-2-hydrazino quinoxaline **3** (3.89g, 0.02 mole), sodium azide (1.3g, 0.02 mole) was added and refluxed for 2 hr in an oil bath. The crude product that separated out was filtered, washed with water and recrystallised from ethanol to furnish the desired compound. The yield of the product was 83% mp. 187-189^{o}C, Mass (m/z): 199.

Synthesis of 1-substituted benzylidene hydrazino-2-tetrazolo [1,5-*a*] quinoxalin–4-yl) derivatives (5a-j)
To an ethanolic solution of 2-hydrazino tetrazolo [1, 5-*a*] quinoxaline **4** (2.01g, 0.01 mole) was refluxed with DMF and various substituted aromatic aldehydes (0.01 mole). The resulting solid products afforded in good yields was recrystallised from methanol and dried.

Spectral Data
1- [(2-hydroxy benzylidene) hydrazino)-2-tetrazolo [1, 5-a] quinoxaline)] **(5a).** Yield 66%, mp. 252-254^{o}C, IR (KBr) cm^{-1}: 3020 (Ar-CH), 3344 (NH), 1618 (N=N), 1561 (C=N), 1523 (C-N), 3525 (-OH); ^{1}HNMR ($CDCl_3$, DMSO-d_6): δ 7.32-8.40 (m, 8H, Ar-H), 11.82 (s, 1H, NH), 8.24 (s, 1H, CH), 8.43 (s, 1H, -OH); Mass (m/z): 305.

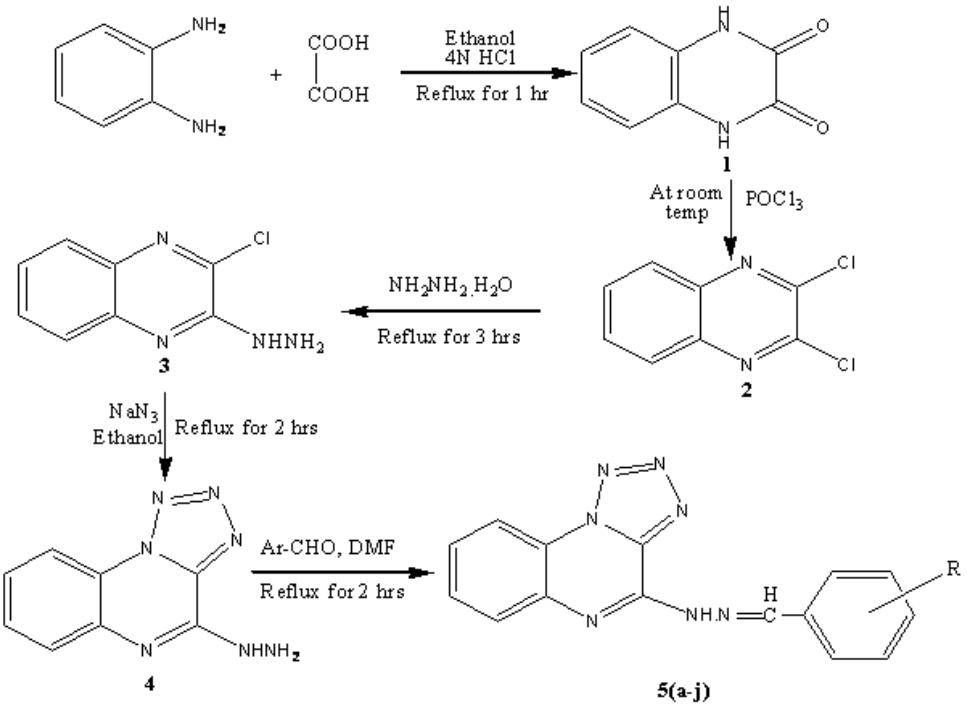
1-[(3-hydroxy benzylidene) hydrazino)-2-tetrazolo [1, 5-a] quinoxaline)] **(5b).** Yield 72%, mp. 293-296^{o}C, IR (KBr) cm^{-1}: 3042 (Ar-CH), 3352 (NH), 1632 (N=N), 1567 (C=N), 1576 (C-N), 3532 (-OH); ^{1}HNMR ($CDCl_3$, DMSO-d_6): δ 7.40-8.62 (m, 8H, Ar-H), 11.64 (s, 1H, NH), 8.32 (s, 1H, CH), 8.43 (s, 1H, -OH); Mass (m/z): 305.

1- [(4-hydroxy benzylidene) hydrazino)-2-tetrazolo [1, 5-a] quinoxaline)] **(5c).** Yield 62%, mp. 263-265^{o}C, IR (KBr) cm^{-1}: 3028 (Ar-CH), 3350 (NH), 1632 (N=N), 1560 (C=N), 1572 (C-N), 3530 (-OH); ^{1}HNMR ($CDCl_3$): DMSO-d_6): δ 7.32-8.20 (m, 8H, Ar-H), 11.63 (s, 1H, NH), 8.28

(s, 1H, CH), 8.43 (s, 1H, -OH); Mass (m/z): 305.

1- [(2-nitro benzylidene) hydrazino)-2-tetrazolo [1, 5-a] quinoxaline)] **(5d).** Yield 85%, mp. 254-258^{o}C, IR (KBr) cm^{-1}: 3028 (Ar-CH), 3420 (NH), 1620 (N=N), 1648 (C=N), 1534 (C-N), 1564 (Ar-NO_2); ^{1}HNMR ($CDCl_3$, DMSO-d_6): δ 7.62-7.83 (m, 8H, Ar-H), 11.42 (s, 1H, NH), 8.76 (s, 1H, CH); Mass (m/z): 334.

Scheme 1



1- [(3-nitro benzylidene) hydrazino)-2-tetrazolo [1, 5-a] quinoxaline)] **(5e).** Yield 88%, mp. 262-265^{o}C, IR (KBr) cm^{-1}: 3032 (Ar-CH), 3428 (NH), 1636 (N=N), 1652 (C=N), 1562 (C-N), 1568 (Ar-NO_2); ^{1}HNMR ($CDCl_3$, DMSO-d_6): δ 7.62-7.74 (m, 8H, Ar-H), 11.52 (s, 1H, NH), 8.82 (s, 1H, CH); Mass (m/z): 334.

1-[(4-nitro benzylidene) hydrazino)-2-tetrazolo [1, 5-a] quinoxaline)] **(5f).** Yield 78%, mp. 296-298^{o}C, IR (KBr) cm^{-1}: 3028 (Ar-CH), 3420 (NH), 1632 (N=N), 1622 (C=N), 1534 (C-N), 1565 (Ar-NO_2); ^{1}HNMR ($CDCl_3$, DMSO-d_6): δ 7.62-7.80 (m, 8H, Ar-H), 11.44 (s, 1H, NH), 8.78 (s, 1H, CH); Mass (m/z): 334.

1- [(2-chloro benzylidene) hydrazino)-2-tetrazolo [1, 5-a] quinoxaline)] **(5g).** Yield 83%, mp. 279-282^{o}C, IR (KBr) cm^{-1}: 3047 (Ar-CH), 3342 (NH), 1620 (N=N), 1648 (C=N), 1146 (C-N), 799 (C-Cl); ^{1}HNMR ($CDCl_3$, DMSO-d_6): δ 7.36-8.24 (m, 8H, Ar-H), 11.74 (s, 1H, NH), 8.86 (s, 1H, CH); Mass (m/z): 323.

1- [(4-chloro benzylidene) hydrazino)-2-tetrazolo [1, 5-a] quinoxaline)] **(5h).** Yield 85%, mp. 307-310^{o}C, IR (KBr) cm^{-1}: 3052 (Ar-CH), 3343 (NH), 1628 (N=N), 1652 (C=N), 1156 (C-N),

796 (C-Cl); ^{1}HNMR ($CDCl_3$, DMSO-d_6): δ 7.34-8.42 (m, 8H, Ar-H), 11.84 (s, 1H, NH), 8.92 (s, 1H, CH); Mass (m/z): 323.

1-[(2-dimethyl amino benzylidene) hydrazino)-2-tetrazolo [1,5-a] quinoxaline)] **(5i).** Yield 87%, mp. 283-287^{o}C, IR (KBr) cm^{-1}: 3056 (Ar-CH), 3348 (NH), 1628 (N=N), 1548 (C=N), 1174 (C-N), ^{1}HNMR ($CDCl_3$, DMSO-d_6): δ 7.23 -8.42 (m, 8H, Ar-H), 12.14 (s, 1H, NH), 8.42 (s, 1H, CH), 3.84 (s, 6H, N $(CH_3)_2$); Mass (m/z): 332.

1-[(3,4,5-trimethoxy benzylidene) hydrazino)-2-tetrazolo [1, 5-a] quinoxaline)] **(5j).** Yield 75%, mp. 317-319^{o}C, IR (KBr) cm^{-1}: 3042 (Ar-CH), 3298 (-NH), 1592 (N=N), 1562 (C=N), 1538 (C-N), 1040 (-C-OCH_3); ^{1}HNMR ($CDCl_3$, DMSO-d_6): δ 7.46 -8.62 (m, 6H, Ar-H), 12.32 (s, 1H, NH), 8.62 (s, 1H, -CH), 3.8 (s, 9H, $(OCH_3)_3$); Mass (m/z): 379.

Biological Screening

Anti-Bacterial activity

All the newly synthesized compounds were subjected to *in vitro* screening against some pathogenic microorganisms *Escherichia coli, Pseudomonas aeruginosa* (Gram negative bacterial strains) and *Staphylococcus aureus, Klebsiella pneumoniae* (Gram positive bacterial strains). The minimum inhibitory concentration (MIC) was determined using tube dilution method [22]. Mueller - Hinton broth was used as culture medium. Sterilized medium was dispensed in each borosilicate glass test tubes. The drug solution was added in order to attain final concentration of 500, 250, 125, 62.5, 31.25, 16.12, 8, 4, 2, 1µg/ml in DMF. Inoculum's of standard suspension (0.1ml of the test organism strain which contains 5 X 10^2 colony forming units /ml) was added to tubes. The tubes were incubated at 37^{o}C for 24 hr and then examined for the presence or absence of growth of the organisms. The lowest concentration which showed no visible growth was taken as an endpoint and Minimum Inhibitory Concentration (MIC) testing results are depicted in Table 2 are the mean of readings obtained in triplicate. Standard known antibiotic such as ofloxacin was used for comparison purpose.

Antifungal activity

The title compounds were investigated at 500, 250, 125, 62.5, 31.25, 16.12, 8, 4, 2, 1 µg/ml concentrations for their *in vitro* antifungal activity using Sabouraud dextrose agar medium. Dimethyl formamide (DMF) was used as solvent for sample preparation. The minimum inhibitory concentration (MIC) was determined and interpreted for *Aspergillus niger* and *Candida albicans* [23]. Sabouraud dextrose agar medium containing griseofulvin as well as control sabouraud dextrose agar medium was inoculated with the microorganisms and incubated at 28^{o}C for 48 hr. The results of activities are tabulated in Table 2 and are are the mean of readings obtained in triplicate.

***In vitro* anti - inflammatory activity** [24]

The human red blood cell membrane stabilization method has used for this study. The blood was collected from healthy human volunteer who has not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsiever's solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10% suspension was made.

Table 2. *In vitro* antimicrobial screening data of 5a-j (MIC in µg/ml)

Compound	*E.coli*	*P.aeruginosa*	*S. aureus*	*K.pneumoniae*	*A.niger*	*C.albicans*
5a	> 500	16.12	125	62.5	125	> 500
5b	> 500	32.25	250	125	250	> 500
5c	> 500	32.25	125	62.5	250	> 500
5d	> 500	2.00	4.00	2.00	1.00	> 500
5e	> 500	16.12	16.12	8.00	8.00	> 500
5f	> 500	4.00	8.00	31.25	2.00	> 500
5g	> 500	8.00	31.25	8.00	31.25	> 500
5h	> 500	8.00	62.5	8.00	62.5	> 500
5i	> 500	125	250	250	500	> 500
5j	> 500	16.12	125	16.12	62.5	> 500
Ofloxacin	2.00	2.00	2.00	2.00	-	-
Griseofulvin	-	-	-	-	8.00	8.00

Table 3. *In vitro* anti-inflammatory screening data of title compounds 5a-j

Compound	Percentage Inhibition at various concentrations			
	50µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml
5a	-	41	44	46
5b	-	45	47	50
5c	-	43	46	48
5d	-	20	22	23
5e	-	23	25	29
5f	-	21	23	24
5g	-	31	35	38
5h	-	34	36	39
5i	-	66	68	71
5j	-	64	65	68
Diclofenac sodium (50µg/ml)	74	-	-	-

The anti-inflammatory activities data depicted in Table 3 are the mean of readings obtained in triplicate. The synthesized compounds at various concentrations (250, 500 and 1000 µg/ml) were prepared using distilled water and to each concentration 1ml of phosphate buffer, 2ml of hyposaline and 0.5ml of HRBC suspension were added. It is incubated at 37^{o}C for 30 min and centrifuged at 3000rpm for 20 min. The hemoglobin content of the supernatant solution was estimated spectrophotometrically at 540 mm. Diclofenac sodium (50µg/ml) was used as a reference standard and a control was prepared by omitting the test compounds. The results of activities are presented in Table 3.

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