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Microwave assisted synthesis of 2-(2-(tetrazolo[1,5-*a*]quinolin-4-yl)-2,3-dihydro-1*H*-1,5-benzodiazepin-4-yl) substituted phenols and evaluation of their antimicrobial activity

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Abstract: A series of new benzodiazepines **4a**–**h** were synthesized by Michael addition of chalcones **3a**–**h** with *o*-phenylenediamine (OPDA) in presence of sodium acetate under conventional heating and microwave irradiation. The structures of the newly synthesized benzodiazepines were established based on IR, ^{1}H- and ^{13}C-NMR and mass spectral data and tested for their antimicrobial activity.

Keywords: Antimicrobial activity; benzodiazepines; chalcones; Michael addition; microwave irradiation; tetrazoloquinoline aldehyde.

INTRODUCTION

The recent literature is enriched with progressive findings concerning the synthesis and pharmacological properties of benzodiazepines. Benzodiazepines; were found to possess a wide range of biological activities and therapeutic uses.[1] In addition to their well-known anxiolytic, anticonvulsant, sedative, and muscle-relaxant activities found in therapeutics,[2] benzodiazepines also exhibit activities as antibiotics,[3] anti-HIV agents[4] and farnesyltransferase inhibitors.[5]

On the other hand, quinoline derivatives are also known to exhibit antiallergic,[6] anticonvulsant,[7] antimicrobial[8] and antimalarial[9] activities. Many chalcone derivatives have been reported to show antimalarial[10] and anticancer[11] activities. Synthesis of quinolinyl chalcones is scarcely reported in the literature whereas chalcones derived from tetrazolo[1,5-*a*]quinoline-4-carboxaldehyde are not reported so far. Moreover, fusion of tetrazole, which is considered as a planar acidic heterocyclic analogue of a carboxylic function, has the ability to increase potency and bioavailability of quinolinyl chalcones. The tetrazole group, which is considered as an analogue to the carboxylic group, as a pharmacophore possesses a wide range of biological activities. Several substituted tetrazoles were shown to

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possess anticonvulsant,[12] anti-inflammatory,[13] CNS depressant,[14] anti-HIV[15] and antifertility[16] properties. Some drugs containing quinoline, chalcone or benzodiazepine moiety, which are readily available on the market, are shown in Fig. S-1 of the Supplementary material to this paper.

Encouraged by the biological activities of benzodiazepines and tetrazolo[1,5-*a*]quinoline moieties and in continuation of on-going endeavors towards the synthesis of biologically potent heterocyclic compounds, the synthesis of new benzodiazepines **4a**–**h** was undertaken.

EXPERIMENTAL

Materials

All used chemicals were obtained commercially, mostly from Sigma–Aldrich, and used without further purification.

Equipment

Melting points were determined by the open capillary method using an electrical melting point apparatus and are uncorrected. Microwave reactions were performed in a MultiSYNTH series microwave system (Milestone). The IR spectra were recorded as KBr pellets on a Shimadzu FT-IR-8400s spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker DPX 400 MHz spectrophotometer using TMS as the internal standard and DMSO-d_6/$CDCl_3$ as solvent. The mass spectra were recorded on a GCMS-QP 1000 EX mass spectrometer and thin layer chromatography (TLC) was performed to check the purity of the compounds, the spot being located under UV light and iodine vapor.

*General procedure for the synthesis of (*E*)-1-(2-hydroxyphenyl)-3-(tetrazolo[1,5-*a*]quinolin-4-yl)prop-2-en-1-ones (**3a**–**h**)*

Conventional method A. To a mixture of a substituted 2-hydroxyacetophenone **1** (0.01 mol) and tetrazolo[1,5-*a*]quinoline-4-carboxaldehyde **2** (0.01 mol) in ethanol (30 mL) was added KOH (0.005 mol) and stirred for overnight at room temperature. The progress of the reaction was monitored by TLC. After completion, the reaction mixture was poured into crushed ice and neutralized with dilute HCl. The thus obtained yellow solid was filtered, washed with water and dried. Recrystallization with methanol afforded the pure chalcones **3a**–**h**. Yields: 56–68 %.

Microwave method B. To a mixture of substituted 2-hydroxyacetophenone **1** (0.01 mol) and tetrazolo[1,5-*a*]quinoline-4-carboxaldehyde **2** (0.01 mol) in ethanol (10 mL) in a 30 mL glass vial equipped with z-cap, was added KOH (0.005 mol) and the mixture was then irradiated for 15–17 min. at 125 °C, using an irradiation power of 180 W. The °progress of the reaction was monitored by TLC. After completion of the reaction, the vial was cooled, diluted with crushed ice and neutralized with dilute HCl. The thus obtained yellow solid was filtered, washed with water and dried. Recrystallization with methanol afforded the pure chalcones **3a**–**h**. Yields: 80–85%.

*General procedure for the synthesis of 2-(2-(tetrazolo[1,5-a]quinolin-4-yl)-2,3-dihydro-1H-1,5-benzodiazepin-4-yl) substituted phenols (**4a**–**h**)*

Conventional method A. To a solution of chalcone **3a**–**h** (0.01 mol) in EtOH (5 mL), containing sodium acetate (0.01 mol), *o*-phenylenediamine (OPDA, 0.01 mol) was added and the reaction mixture was heated at 80–90 °C for 2–3 h. The progress of the reaction was monitored by TLC. After the completion of the reaction, ice water was added. A solid product

separated hat was filtered, washed with water and dried. The crude products were recrystallized from MeOH: $CHCl_3$ (1:1). Yields: 60–70 %.

Microwave method B. To a solution of chalcones **3a**–**h** (0.01 mol) in EtOH (5 mL), containing sodium acetate (0.01 mol) in a 10 mL glass vial equipped with a cap, OPDA (0.01 mol) was added and the mixture was then irradiated for 2–4 min. at 130 °C, using an irradiation power of 180 W. The progress of the reaction was monitored by TLC for each 30 s of time interval. After the completion of the reaction, ice water was added. A solid product separated was filtered, washed with water and dried. Recrystallization from MeOH:$CHCl_3$ (1:1). Yields: 80–86 %.

Spectral data are given in the Supplementary material to this paper.

Biological assay

Antibacterial activity. All the synthesized compounds **3a**–**h** and **4a**–**h** were screened for their antibacterial activity against Gram-positive (*Staphylococcus aureus* and *Bacillus facealis*) and Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*). The bacterial cultures were grown in nutrient agar media and subcultured for better growth (log phase cultures) in a liquid nutrient broth medium and further subcultured onto Petri plates for the experiments.

Both cultures were diluted with sterilized saline to bring the final size of the inoculum to approximately 10^5–10^6 CFU mL^{-1}. The compounds were diluted in acetone, DMSO and diethyl ether for the biological assays. Of the three solvents, diethyl ether was taken as being the best.

The bacterial culture inoculum was placed on the media and incubated at 37 °C for 24 h to 48 h along with the chemical discs dipped and placed over the media. The zones of bacterial growth inhibition were measured using the diameter of the zone as a unit to measure the antibacterial activity. All the experiments were performed in triplicate and the results are expressed as zone of inhibition in mm.

The results were compared with the activity of the standard antibiotic ciproflaxin (20 and 40 μg mL^{-1}). For disc diffusion method, a test compound was introduced into the disc and then allowed to dry. Once the disc was completely saturated with the test compound, it was introduced into the upper layer of the medium containing the bacterial inoculum. The Petri dishes were incubated overnight at 37 °C for 24 h. Bioactivity was determined by measuring the diameter of the inhibition zones (*DIZ*) in mm. The antibacterial activity was evaluated and the results are presented in Table I.

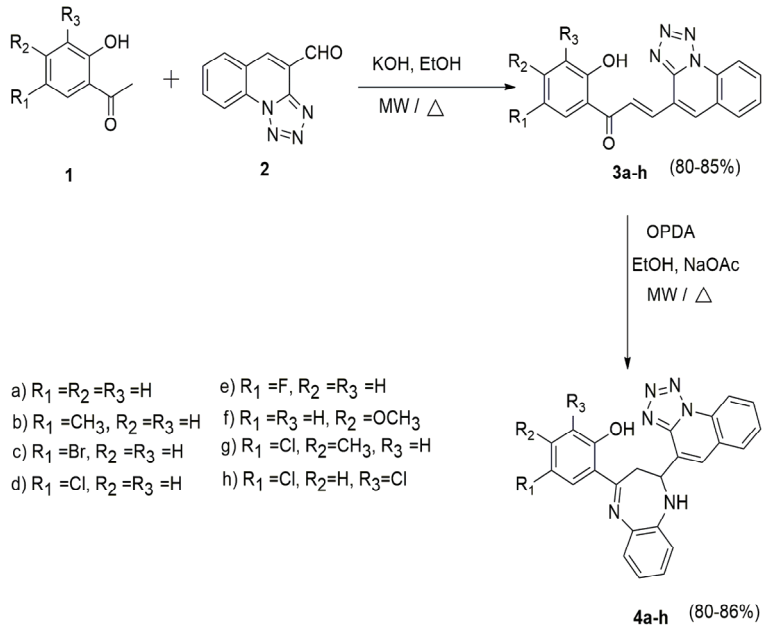
Antifungal activity. The antifungal activities of the synthesized compounds **3a–h** and **4a–h** were tested against three pathogenic fungi (*A. niger*, *A. flavus* and *Fusarium oxysporum*) by the poison plate technique. The test compounds were dissolved in diethyl ether (10 mL) before mixing with Potato Dextrose Agar medium (PDA, 90 mL). The final concentration of compounds in the medium was 50 μg mL. The above-mentioned types of fungi were incubated in PDA at 25±1 °C for 3–4 days to obtain good mycelium growth for the antifungal assay, then a mycelia disk of approximately 0.45 cm diameter cut from the culture medium was picked out with a sterilized inoculation needle and inoculated in the center of PDA plate. The inoculated plates were incubated at 25±1 °C for 5 days. Diethyl ether in sterilized distilled water was used as control, while hymexazole was used as positive control for all the treatment, three replicates were performed. The radial growth of the fungal colonies was measured on the fourth day and the data were statistically analyzed. The *in vitro* inhibition effects of the test compounds on the fungi were calculated by the given formula $CV = A–B/A$, where A represents the diameter of fungi growth on untreated PDA, B represents the diameter of fungi on

treated PDA, and CV represents the rate of inhibition. The antifungal activity was evaluated and the results are presented in Table II and compared with that of the standard drug amphotericin-B.

RESULTS

In recent years, reports on microwave assisted synthesis revealed that it is a safe, rapid, economic and environmental friendly procedure.[17] Owing to increased regulatory pressure in research and industry, tremendous efforts have been made to reduce the amount of pollutants produced, including organic solvents in chemical synthesis. To enforce such practices, the discovery and invention of new synthetic methods are required.

The synthesis of new derivatives of benzodiazepines was performed as outlined in Scheme 1. The chalcones were prepared by reacting substituted 2-hydroxyacetophenones **1a**–**h** with tetrazolo[1,5-*a*]quinoline-4-carboxaldehyde (**2**) in the presence of KOH by conventional and microwave irradiation using the Claisen–Schmidt condensation.[17] The reaction of (*E*)-1-(2-hydroxy substituted phenyl)-3-(tetrazolo[1,5-*a*]quinolin-4-yl)prop-2-en-1-ones **3a**–**h** with *o*-phenylenediamine in EtOH was performed by conventional as well as microwave irradiation in the presence of sodium acetate to give 2-(2-(tetrazolo[1,5-*a*]quinolin-4--yl)-2,3-dihydro-1*H*-1,5-benzodiazepin-4-yl) substituted phenols **4a**–**h**. The formation of benzodiazepines **4a–h** was confirmed by IR, NMR and mass analyses.



1 2 **3a-h** (80-85%) **4a-h** (80-86%)

Scheme 1. Synthesis of 2-(2-(tetrazolo[1,5-*a*]quinolin-4-yl)-2,3-dihydro-1*H*-1,5-benzodiazepin-4-yl) substituted phenols **4a**–**h**.

It was found that the synthesis of benzodiazepines **4a**–**h** by the conventional method took a longer time (2–3 h) and gave lower yields, when compared to microwave irradiation technique in which the reaction proceeded smoothly with excellent yields, within a few minutes (2–3 min).

The ^{1}H-NMR spectra of benzodiazepine **4a** displayed three characteristic signals in the aliphatic region corresponding to the diastereotopic protons (H_A, H_B and H_X). The H_A proton, which is *cis* to H_X, resonated upfield in the δ range 3.38–3.46 ppm as doublet of doublets (*dd*), while the H_B proton, which is *trans* to H_X, resonated downfield in the δ range of 3.80–3.88 ppm (*dd*). The H_X proton, which is vicinal to two methylene protons (H_A and H_B), also resonated as a doublet of doublets in the δ region 6.16–6.24 ppm.

The cyclization of chalcones into benzodiazepines was further supported by the ^{13}C-NMR spectrum of **4a**, in which the CH_2 and CH carbons resonated at δ 32.12 and 65.40 ppm, respectively.[18] These values are in close agreement with the reported values for benzodiazepine carbons CH_2 and CH. The combination of ^{1}H-NMR and ^{13}C-NMR data provided strong evidence in support of the structures assigned to the benzodiazepine derivatives.

The mass spectrum of **4a** showed a molecular ion peak at *m*/*z* 407 $[M+H]^+$. The NMR and MS data were found to be satisfactory for the structures assigned to compounds **4a**–**h**.

Antibacterial activity

All the synthesized compounds **3a**–**h** and **4a**–**h** were screened for their antibacterial activity against Gram-positive (*S. aureus* and *B. faecalis*) and Gram-negative bacterial strains (*E. coli* and *K. pneumoniae*) at 20 and 40 μg mL^{-1} concentrations. The zone of inhibition (in mm) was compared with those of the standard drug ciprofloxacin. All the compounds showed relatively better activity against Gram-positive bacterial strains than Gram-negative bacterial strains (Table I). The compounds **3a**, **3b**, **3f**, **4a**, **4b** and **4f** showed promising activity against Gram-positive bacterial strains. Compounds **4a** (R_1, R_2, R_3 = H), **4b** (R_1 = CH_3, R_2, R_3 = H) and **4f** (R_1, R_2 = H, R_3 = OCH_3) showed maximum zones of inhibition. Compound **4f** exhibited the most potent antibacterial activity against Gram-negative bacterial strains. The remaining compounds showed moderate activity against Gram-negative bacterial strains. It could be concluded that the compounds with an electron donating methyl and methoxy groups displayed a more potent activity.

Antifungal activity

The antifungal activities of the synthesized compounds **3a**–**h** and **4a**–**h** were tested against three pathogenic fungi (*A. niger*, *A. flavus* and *F. oxysporum*) at 50 μg mL^{-1} concentration. The zones of inhibition (in mm) were compared with those

TABLE I. Antibacterial activity of **3a**–**h** and **4a**–**h**

Compound	Gram-positive bacterial strains				Gram-negative bacterial strains			
	B. faecalis		*S. aureus*		*K. pneumoniae*		*E. coli*	
	c / μg mL^{-1}							
	20	40	20	40	20	40	20	40
3a	15	28	14	27	12	23	11	20
3b	16	30	15	26	8	16	10	21
3c	13	22	11	20	9	16	9	17
3d	10	19	10	15	9	19	6	13
3e	9	17	9	15	8	13	6	12
3f	16	28	16	28	7	15	8	17
3g	9	15	9	15	10	19	9	14
3h	11	19	13	25	7	15	5	10
4a	17	30	16	29	9	17	9	16
4b	17	31	15	28	11	22	10	19
4c	11	20	7	15	9	12	5	12
4d	10	19	9	20	14	22	8	17
4e	11	20	10	17	7	16	8	17
4f	16	30	15	27	22	35	16	34
4g	9	17	8	13	13	20	5	10
4h	11	23	12	18	24	34	17	33
Ciprofloxacin	16	30	15	28	23	35	18	35

of the standard drug amphotericin-B (Table II). All the compounds showed moderate to good activity against the tested fungal strains. Compounds **3a**, **3f**, **4c** and **4h** showed maximum activity against *A. niger*, compounds **3c** and **4c** were active against *A. flavus* and compound **4f** showed promising activity against *F. oxysporum*. The antifungal activity results revealed the electron donating methyl and methoxy groups played a significant role for the inhibitory potency of the compounds against the fungal strains.

TABLE II. Antifungal activity of **3a**–**h** and **4a**–**h**; c = 50 μg mL^{-1}

Compound	Fungi		
	A. niger	*A. flavus*	*F. oxysporum*
3a	15	6	7
3b	8	7	8
3c	6	14	5
3d	8	9	6
3e	3	4	8
3f	15	5	3
3g	10	2	9
3h	6	5	2
4a	11	10	8
4b	10	9	7
4c	16	15	10
4d	6	10	8

TABLE II. Continued

Compound	Fungi		
	A. niger	*A. flavus*	*F. oxysporum*
4e	9	8	6
4f	10	10	15
4g	7	8	4
4h	15	10	11
Amphotericin-B	16	15	16

CONCLUSIONS

An easy, high yielding, convenient method for the synthesis of 2-(2-(tetrazolo[1,5-*a*]quinolin-4-yl)-2,3-dihydro-1*H*-1,5-benzodiazepin-4-yl)substituted phenols **4a**–**h** from (*E*)-1-(2-hydroxy substituted phenyl)-3-(tetrazolo[1,5-*a*]quinolin-4-yl)prop-2-en-1-ones **3a**–**h** in conventional and microwave irradiation routes is reported. The microwave irradiation process proved to be a simple environmentally friendly technique with high yields and high rate of acceleration. All the new compounds were screened for their antimicrobial activity. It was observed that compounds **3a**, **3b**, **3f**, **4a**, **4b**, and **4f** exhibited broad spectrum of antibacterial activity, and compounds **3a**, **3f**, **4c**, **4f** and **4h** shown broad spectrum of antifungal activity against all the tested strains compared to the standard drugs with their respective concentrations.

SUPPLEMENTARY MATERIAL

Spectral data of the synthesized compounds are available electronically from http://www.sbd.org.rs/JSCS/ or from the corresponding author on request.

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ИЗВОД

СИНТЕЗА 2-(2-(ТЕТРАЗОЛО[1,5-*A*]КИНОЛИН-4-ИЛ)-2,3-ДИХИДРО-1*H*-1,5-БЕНЗОДИАЗЕПИН-4-ИЛ) СУПСТИТУИСАНИХ ФЕНОЛА ПОД МИКРОТАЛАСИМА И ИСПИТИВАЊЕ ЊИХОВЕ АНТИМИКРОБНЕ АКТИВНОСТИ

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Синтетисана је серија бензодиазепина **4a**–**h** реакцијом Мајклове адиције, полазећи од халкона **3a**–**h** и *o*-фенилендиамина (OPDA), у присуству натријум-ацетата, под условима класичног термичког загревања и озрачивањем микроталасима. Структура синтетисаних бензодиазепина **4a**–**h** утврђена је на основу података IC и NMR (^{1}H- и ^{13}C-) спектроскопије и масене спектрометрије и испитана је антимикробна активност синтетисаних једињења.

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