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Microwave-assisted multi-component synthesis of indol-3-yl substituted pyrano[2,3-*c*]pyrazoles and their antimicrobial activity

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Abstract: A series of pyrano[2,3-*c*]pyrazole derivatives of indole was synthesized by multi-component reactions using the conventional and microwave irradiation approach. Particularly valuable features of this method include high yield, broad substrate scope, shorter reaction times and straightforward procedure. Antimicrobial screening of the synthesized derivatives against eight human pathogens, namely *Bacillus subtilis*, *Clostridium tetani*, *Streptococcus pneumoniae*, *Salmonella typhi*, *Vibrio cholerae*, *Escherichia coli*, *Aspergillus fumigatus* and *Candida albicans*, was realized by employing the broth microdilution minimum inhibition concentration method, as recommended by National Committee for Clinical Laboratory Standards (NCCLS).

Keywords: indole; pyranopyrazole; multi-component reaction; microwave irradiation; antimicrobial activity.

INTRODUCTION

Indole derivatives are a topic of substantial research interest in contemporary heterocyclic and medicinal chemistry due to their great significance in the view of their *i*) occurrence in nature as a prominent sub-structure of a large number of alkaloids[1] and *ii*) wide-ranging biological activities, which include antimicrobial,[2] antitubercular,[3] anticancer,[4] antioxidant,[5] antiviral,[6] antimalarial,[7] *etc*. Moreover, the 4*H*-pyran nucleus is a fertile source of biologically important molecules possessing a wide spectrum of pharmacological activities, such as antimicrobial,[8] antiviral,[9] mutagenic,[10] sex pheromone,[11] antitumour,[12] cancer therapy[13] and central nervous system activity.[14]

The conventional procedures for the synthesis of indole-based pyrano[2,3--*c*]pyrazole are not satisfactory with regards to operational simplicity, effective-

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ness and yield. An alternative synthetic approach is microwave irradiation.[15] In recent years, microwave irradiation has been demonstrated not only to dramatically accelerate many organic reactions, but also to improve yield and selectivity. The optimizations of reaction strategies[16] *via* three-component one-pot conventional synthesis and four-component microwave assisted synthesis were aimed at improving the yield and selectivity, and reducing the use of hazardous organic bases, such as piperidine. A literature survey revealed that a number of pyranopyrazole derivatives have been synthesized using various aldehydes[8] but not a single report existed in which 2-phenyl-1*H*-indole-3-carbaldehyde was used.

It must be emphasized that a combination of the pyranopyrazole template with other heterocycles is a well-known approach for the build-up of drug-like molecules, which allows the achievement of new pharmacological profiles, action strengthening or toxicity lowering. As part of ongoing research aimed at the discovery of new active antimicrobial compounds,[17] in this work an attempt was made to study the influence of the combination of the pyranopyrazole moiety and the 2-phenyl-1*H*-indole scaffold on antimicrobial effect of the resulting compounds. Hence, herein, the synthesis of some new derivatives of pyranopyrazole **5a**–**h** *via* the multi-component reaction approach under microwave irradiation is reported. The newly synthesized compounds were characterized by elemental analysis, and FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectrometry. All the compounds were screened for their *in vitro* antimicrobial activity against a representative panel of bacteria and fungi using the broth microdilution *MIC* (minimum inhibitory concentration) method.[18]

RESULTS AND DISCUSSION

Chemistry

The title derivatives were synthesized in two ways, *i.e.*, three-component piperidine catalyzed reaction and microwave-assisted four-component one pot reaction catalyzed by NaOH. The comparative study based on the optimization of both methods is depicted in Table I, which indicates that the reactions were efficiently promoted by microwave irradiation. The reaction time was strikingly shortened from 2–2.5 h (under traditional heating conditions) to 5–6 min (under microwave irradiation) and quantitative yields were obtained.

The key intermediates substituted 2-phenyl-1*H*-indole-3-carboxaldehydes **1a**–**h** were prepared by the Vilsmeier–Haack Reaction of 2-phenyl-1*H*-indole according to a literature procedure.[19] The title compounds **5a**–**h** were synthesized by microwave-assisted four-component synthesis with good yields (73–84 %) (Table I).

The reaction occurred *via* initial *in situ* formation of the heterylidenenitriles (Scheme 1), containing the electron-poor C=C double bond, from the Knoevenagel condensation between 2-phenyl-1*H*-indole-3-carbaldehyde **1a**–**h** and malono-

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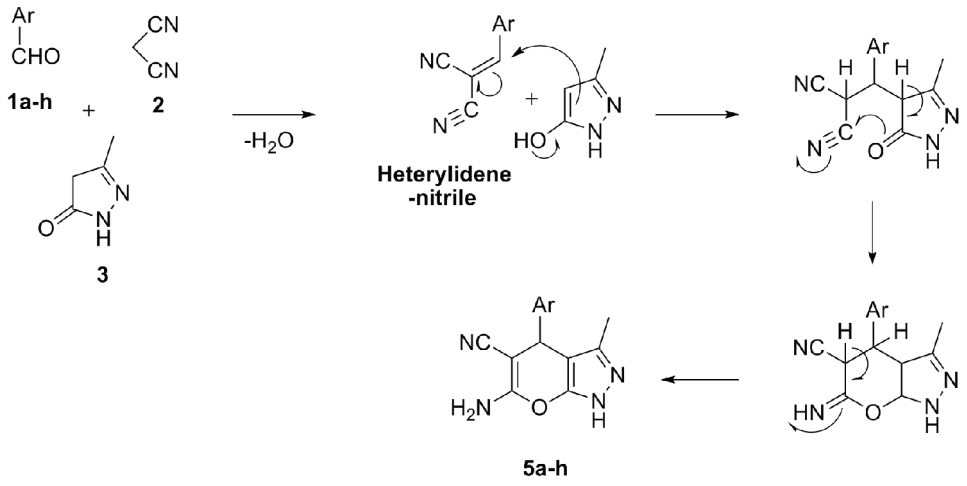
nitrile **2** by loss of water molecules. Finally, Michael addition of 3-methyl-1*H*-pyrazol-5(4*H*)-one **3** to the initially formed unsaturated nitrile, *i.e.*, nucleophilic attack of the hydroxyl moiety on the cyano moiety afforded the cyclised 4*H*-pyran derivatives **5a**–**h**. A similar mechanism maybe operative in the four-component reaction.

TABLE I. Comparison of microwave and conventional method and physical data of the compounds **5a**–**h**

Compd.	R	Microwave		Conventional		M.p. / °C (after crystallization)
		Time min	Isolated yield %	Time h	Isolated yield %	
5a	H	5	74	2	65	206–208
5b	CH_3		73		61	201–203
5c	OCH_3		68		59	200
5d	Cl	6	79	2.5	70	214–216
5e	Br		82		73	204–206
5f	F	5	80	2	69	210
5g	NO_2		76		67	209–211
5h	SO_2CH_3		84		74	214



Scheme 1. Plausible mechanistic pathway of the synthesis of pyranopyrazole derivatives **5a**–**h**.

The structures of all the newly synthesized compounds were established by ^{1}H-NMR, ^{13}C-NMR, FT-IR and mass spectrometry, and elemental analysis. The IR spectrum of compound **5c** exhibited characteristic absorption bands at 3415 and 3320 cm^{-1} (asym. & sym. str.) for –NH_2 and at 2195 (C≡N stretching) and 1250 cm^{-1} (asym. str. of cyclic C–O–C ether). The ^{1}H-NMR spectrum of com-

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pound **5c** indicated the presence of one singlet peak at δ 4.95 ppm of the –CH proton and the disappearance of a singlet at δ 10.50 ppm of –CHO, which clearly confirmed the cyclization of the Knoevenagel intermediate. The singlet at δ 6.82 ppm arose from the NH_2 protons of the pyran ring. All the aromatic protons resonated as multiplets at δ 7.03–7.60 ppm. The singlets at δ 1.54 and 11.92 ppm are due to the presence of the methyl and secondary amine of the fused pyrazole ring, respectively. The singlet peaks at δ 3.08 and δ 11.11 ppm are due to OCH_3 and the secondary amine of the indole ring, respectively. The ^{13}C-NMR spectrum of compound **5c** exhibited a distinctive signal at δ 9.87 ppm for the methyl of the fused pyrazole ring and at δ 27.52 ppm for the C4 of the pyran ring. All the aromatic carbons and C≡N of **5c** showed signals at δ 111.76–155.27 ppm. Moreover, distinctive signals at δ 161.32 ppm for C6 (C–NH_2), δ 55.69 ppm for OCH_3, δ 57.23 ppm for C5 **C**–C≡N), δ 97.88 ppm for C3a and δ 159.51 ppm for C7a in the ^{13}C-NMR spectrum confirmed the structure of **5c**. Furthermore, the structures of all the new compounds were confirmed by mass spectral studies. The mass spectra detected the expected molecular ion signals corresponding to respective molecular formula of the synthesized compounds. The mass spectrum of compound **5c** gave a molecular ion peak at 398 (M^++1), corresponding to the molecular formula $C_{23}H_{19}N_5O_2$. The elemental analysis values and the mass spectral data are in good agreement with the theoretical data.

The analytic and spectroscopic data of all the synthesized compounds are given in the Supplementary Material to this paper together with representative spectra of **5e** and **5f**, the two compounds showing outstanding and excellent antibacterial activity, respectively.

Biological evaluation

The results of the antimicrobial activity screening are presented in Table II. Examination of the data revealed that the majority of the compounds showed good antibacterial and antifungal activity when compared with ampicillin, nystatin and griseofulvin. Compound **5f** (R = F) was found to be exceedingly potent against most of the employed strains.

In particular, compound **5f** (R = F) showed outstanding activity (*MIC* 62.5 μg mL^{-1}) against the Gram-negative bacteria *Escherichia coli*, as compared to the standard ampicillin (*MIC* 100 μg mL^{-1}) and compound **5e** (R = Br) displayed excellent activity (*MIC* 125 μg mL^{-1}) against the Gram-positive bacteria *Bacillus subtilis*, as compared to the standard ampicillin (*MIC* 250 μg mL^{-1}). Compounds **5b** (R = CH_3), **5d** (R = Cl), **5f** (R = F) and **5h** (R = NO_2) against *B. subtilis* (*MIC* 200 μg mL^{-1}) and compounds **5c** (R = OCH_3), **5f** (R = F) and **5h** (R = NO_2) against *Clostridium tetani* (*MIC* 200 μg mL^{-1}) were found significantly active in comparison to ampicillin (*MIC* 250 μg mL^{-1}). Against fungal pathogen *Candida albicans*, compounds **5b** (R = CH_3) and **5f** (R = F) were found to possess better

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activity (*MIC* 250 μg mL^{-1}) as compared to the standard griseofulvin (*MIC* 500 μg mL^{-1}).

TABLE II. Antimicrobial activity of the compounds **5a**–**h** (minimum inhibitory concentration, *MIC* / μg L^{-1}); *S.p., S. pneumoniae*; *C.t., C. tetani*; *B.s., B. subtilis*; *S.t., S. typhi*; *V.c., V. cholerae*; *E.c., E. coli*; *A.f., A. fumigatus*; *C.a., C. albicans*; "–" represents "not tested"

Compound	Gram-positive bacteria			Gram-negative bacteria			Fungi	
	B.s.	*C.t.*	*S.p.*	*E.c.*	*S.t.*	*V.c.*	*A.f.*	*C.a.*
	MTCC							
	441	449	1936	443	98	3906	3008	227
5a (R = H)	250	250	200	250	200	500	1000	500
5b (R = CH_3)	200	500	200	500	500	200	>1000	250
5c (R = OCH_3)	250	200	250	200	200	200	>1000	>1000
5d (R = Cl)	200	500	200	500	500	250	1000	1000
5e (R = Br)	125	250	250	250	250	200	250	500
5f (R = F)	200	200	200	62.5	200	250	1000	250
5g (R = SO_2CH_3)	500	250	250	250	250	250	1000	1000
5h (R = NO_2)	200	200	200	200	250	200	1000	1000
Ampicillin	250	250	100	100	100	100	–	–
Griseofulvin	–	–	–	–	–	–	100	500
Nystatin	–	–	–	–	–	–	100	100

Compounds **5a** (R = H) and **5c** (R = OCH_3) against *B. subtilis*, and compounds **5a** (R = H), **5e** (R = Br) and **5g** (R = SO_2CH_3) against *C. tetani* were found to be equipotent to ampicillin (*MIC* 250 μg mL^{-1}). In the case of the fungal pathogen *C. albicans*, compounds **5a** (R = H) and **5e** (R = Br) were equally active to griseofulvin (*MIC* 500 μg mL^{-1}). Unfortunately, none of the synthesized compounds were found sufficiently potent to inhibit *Streptococcus pneumoniae*, *Salmonella typhi*, *Vibrio cholerae* or the fungal pathogen *Aspergillus fumigatus*.

The investigation of the structure–activity relationship of the antibacterial screening revealed that the compound with 4-fluorophenyl ring at the 2-position of the indole nucleus gave excellent results towards *E. coli*. Whereas, towards *B. subtilis*, the compounds with 4-methyl-, chloro-, bromo-, fluoro and nitro-phenyl ring substitutions were found to be highly active, while compounds with a 4-methoxy substituted and an unsubstituted phenyl ring showed equal activity to that of ampicillin. Against *C. tetani*, compounds carrying a 4-methoxy, fluoro and nitro phenyl ring were found to be more potent than ampicillin, while the compounds with 4-bromo, methylsulphonyl and unsubstituted phenyl ring and ampicillin gave equal results. Antifungal evaluation showed that 4-methyl and fluoro phenyl ring containing compounds were the most potent, while compounds with 4-bromo and unsubstituted phenyl ring displayed similar activity to griseofulvin

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against fungal species *C. albicans*. Except the nitro substituted title compound, all derivatives displayed excellent antimicrobial activity.

Reviewing and comparing the activity data, it is worthy to mention that the antimicrobial activity of the target compounds depended not only on the bicyclic heteroaromatic pharmacophore, but also on the nature of the peripheral substituents.

EXPERIMENTAL

Materials, instruments and methods

Required acetophenone, phenylhydrazine, polyphosphoric acid, phosphorous oxychloride, ethyl acetoacetate and hydrazine hydrate were obtained commercially. Solvents were purified and dried before use. The microwave-assisted reactions are conducted in a "RAGA's modified electromagnetic microwave system" whereby the microwaves were generated by a magnetron at a frequency of 2450 MHz having adjustable output power levels, *i.e.*, 10 levels from 140 to 700 W and with an individual sensor for temperature control (a fibre optic was used as the individual sensor for temperature control) with the attachment of a reflux condenser under constant stirring (thus avoiding the risk of high pressure development). All melting points were taken in open capillaries and are uncorrected. Thin-layer chromatography (TLC, on aluminium plates pre-coated with silica gel, $^{60}F_{254}$, 0.25 mm thickness) (Merck, Darmstadt, Germany) was used for monitoring the progress of all reactions, and the purity and homogeneity of the synthesized compounds; the eluent was 5:5 hexane:ethyl acetate and UV radiation and/or iodine were used as the visualizing agents. Elemental analysis (% C, H, N) was realised using a Perkin-Elmer 2400 series-II elemental analyzer (Perkin-Elmer, USA) and the values for all compounds are within ±0.4 % of the theoretical values. The IR spectra were recorded in KBr on a Perkin-Elmer Spectrum GX FT-IR Spectrophotometer (Perkin-Elmer, USA) and only the characteristic peaks are reported in cm^{-1}. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded in DMSO-d_6 on a Bruker Avance 400F (MHz) spectrometer (Bruker Scientific Corporation Ltd., Switzerland) using the solvent peak as an internal standard at 400 and 100 MHz, respectively. Chemical shifts are reported in parts per million (ppm). Mass spectra were scanned on a Shimadzu LCMS 2010 spectrometer (Shimadzu, Tokyo, Japan).

*General procedures for the synthesis of 6-amino-4-(2-(4-(un)-substituted phenyl)-1*H*-indol-3--yl)-3-methyl-1,4-dihydropyrano[2,3-*c*]pyrazole-5-carbonitriles **5a**–**h***

Method a: three-component one pot reaction. 2-Phenyl-1*H*-indole-3-carbaldehydes **1a**–**h** (1 mmol), malononitrile **2** (1 mmol), 3-methyl-1*H*-pyrazol-5(4*H*)-one **3** (1 mmol) and ethanol (15 mL) containing piperidine (0.1 mmol) were charged into a 100-mL round bottom flask with mechanical stirrer and condenser. The reaction mixture was slowly heated and refluxed for 2–2.5 h. After completion of the reaction (checked by TLC), the reaction mixture was cooled to room temperature and the solid that separated was filtered and washed with equimolar mixture of chloroform and methanol to obtain the pure compounds.

Method b: four-component microwave irradiation method. 2-Phenyl-1*H*-indole-3-carbaldehydes **1a**–**h** (1 mmol), malononitrile **2** (1 mmol), ethyl acetoacetate **4** (1 mmol), hydrazine hydrate **6** (1.1 mmol) and NaOH (5 mol %) were thoroughly mixed in ethanol (10 mL). The mixture was irradiated for 5–6 min at 280 W output power. After the completion of reaction (checked by TLC), the reaction mixture was cooled to room temperature and the solid that separated was filtered and washed with equimolar mixture of chloroform and methanol to obtain the pure compounds.

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The physical data of compounds are reported in Table I.

Antimicrobial activity

All the employed glass equipment was sterilized before use. Antimicrobial activity of all the synthesized compounds was carried out by the broth microdilution method. Mueller Hinton broth was used as the nutrient medium to grow and dilute the compound suspension for the test bacteria and Sabouraud dextrose broth was used for fungal nutrition. The inoculum size for the test strain was adjusted to 10^8 CFU (colony forming unit) per milliliter by comparing the turbidity. The strains used for the activity were procured from (MTCC – Microbial Type Culture Collection) Institute of Microbial Technology, Chandigarh. Each synthesized compound was diluted to obtain a concentration of 2000 μg mL^{-1}, as the stock solutions. The results were recorded in the form of primary and secondary screening. Compounds **5a**–**h** were screened for their antimicrobial activity against a representative panel of bacteria and fungi at concentrations of 1000, 500 and 250 μg mL^{-1} as the primary screening. DMSO was used as the vehicle to obtain the desired test concentrations of the compounds. The compounds found to be active in the primary screening were further screened in a second set of dilution at concentrations of 200, 100, 62.5 and 50 μg mL^{-1}. 10 μL suspensions from each well were further inoculated and growth was noted after 24 and 48 h. The lowest concentration that allowed no visible growth (turbidity) of the micro-organisms after the spot subculture was considered as the *MIC* for each compound. In the present study, ampicillin was used as standard antibacterial drug, whereas griseofulvin and nystatin were used as standard antifungal drugs. The protocols are summarized in Table II.

CONCLUSIONS

Rapid, simple, and efficient method has been developed for the synthesis of some new indole based pyrano[2,3-*c*]pyrazole derivatives under microwave irradiation conditions in the presence of the non-hazardous base NaOH. The engaged synthetic strategy allows the construction of a relatively complicated nitrogen and oxygen carrying heterocyclic system as well as the introduction of various (hetero) aromatic substitutions into 4-position of the 4*H*-pyran system. It can be concluded from antimicrobial screening against a panel of human pathogens, that most of the synthesized 4*H*-pyran derivatives were highly active against *B. subtilis* and *C. tetani* as well as against *C. albicans* compared to the standard drugs. It is worth mentioning that minor changes in molecular structure of these compounds profoundly influenced the activity. The present study throws light on the identification of this new structural class as antimicrobials, which could be of interest for further detailed preclinical investigations.

SUPPLEMENTARY MATERIAL

Analytical and spectral data of the synthesized compound are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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И З В О Д

СИНТЕЗА 3-ИНДОЛИЛ СУПСТИТУИСАНИХ ПИРАНО[2,3-*c*]ПИРАЗОЛА ПОД ДЕЈСТВОМ МИКРОТАЛАСА И ЊИХОВА АНТИМИКРОБНА АКТИВНОСТ

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Серија деривата пирано[2,3-*c*]пиразола синтетисана је конвенционалним поступцима и под дејством микроталаса. Поступак са микроталасима карактеришу висок принос, широк опсег супстрата који могу да се користе, краће реакционо време и директан поступак. Извршено је тестирање антимикробне активности синтетисаних једињења према осам патогена *Bacillus subtilis*, *Clostridium tetani*, *Streptococcus pneumoniae*, *Salmonella typhi*, *Vibrio cholerae*, *Escherichia coli*, *Aspergillus fumigatus* и *Candida albicans* поступком микроразблаживања у бујону према препоруци NCCLS.

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