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Synthesis and characterization of new 2-amino-4-(3,4-dihydro--7-methoxy-2,2-dimethyl-2*H*-benzopyran-6-yl)-6-(substituted phenyl)pyrimidines and their bioevaluation

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Abstract: A series of eight novel 2-amino-4-(3,4-dihydro-7-methoxy-2,2-dimethyl-2*H*-benzopyran-6-yl)-6-(substituted phenyl)pyrimidines were designed and synthesized by utilizing benzene-1,3-diol as the starting material. The structures of the isolated products (**6a**–**h**) were established through ^{1}H-NMR, ^{13}C-NMR, and FT-IR spectroscopic techniques and elemental analysis. The antimicrobial properties of the synthesized compounds were screened against gram positive *(Bacillus pumilus*, *Bacillus subtilis* and *Streptococcus faecalis*), gram negative bacteria (*Enterococcus faecalis*, *Escherchia coli* and *Proteus vulgaris)* and fungi *(Pencillium expansum, Aspergillus niger* and *Candida albicans)* using ampicillin and ketoconazole as reference compounds. Most of the compounds showed moderate to high antibacterial and antifungal activities against the studied strains, with inhibition zones between 8 and 30 mm.

Keywords: benzopyran; chalcones; Claisen–Schmidt condensation; 2-amino pyrimidine derivatives; antimicrobial activity.

INTRODUCTION

Antibiotics revolutionized medical care in the 20th century. However, the emergence of super bugs, *i.e.*, bacteria that resist the effects of the most powerful antibiotics, are posing a great challenge to the field of medicine.[1] In this sense, nitrogen-containing heterocycles are an important class of compounds in medicinal chemistry.[2] As pyrimidines seem to possess a wide spectrum of biological activity,[3] there has been considerable interest in the development of preparative methods for the production of pyrimidines.

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On the other hand, pyrimidines, being an integral part of DNA and RNA, exhibit diverse pharmacological properties as effective bactericide, fungicide, viricide, insecticide and meticide,[4] antitubercular,[5] antineoplastic,[6] antitumor,[7] anti-inflammatory,[8] diuretic,[9] antimalarial,[10] cardiovascular,[11] calcium channel blockers[12] and many other classes of chemotherapeutic agents.[13]

The benzopyran ring occurs frequently in natural products. However 3,4-dihydro-2*H*-benzopyrans occur rarely in nature but are useful degradation products of a large number of natural products.[14] They have been reported to show marked pharmacological activities.[15] The biological activity of many of the naturally occurring compounds which incorporate a benzopyran ring has resulted in several applications of various levels of saturation and oxidation in synthesis.[16] Due to the various biological functions associated with this skeleton, it has been frequently employed as a key scaffold in drug research.[17]

In view of these observations and in continuation of our work[18] on biologically active heterocycles and their increasing importance in pharmaceutical and biological fields, it was considered of interest to synthesize some new chemical entities incorporating two active pharmacophores in a single molecular framework and to evaluate their biological activities. In this respect, eight novel 2-amino-4-(3,4-dihydro-7-methoxy-2,2-dimethyl-2*H*-benzopyran-6-yl)-6-(substituted phenyl)pyrimidines (**6a**–**h**) were synthesized *via* benzopyranylchalcones and the antimicrobial activities of the compact structures were screened.

EXPERIMENTAL

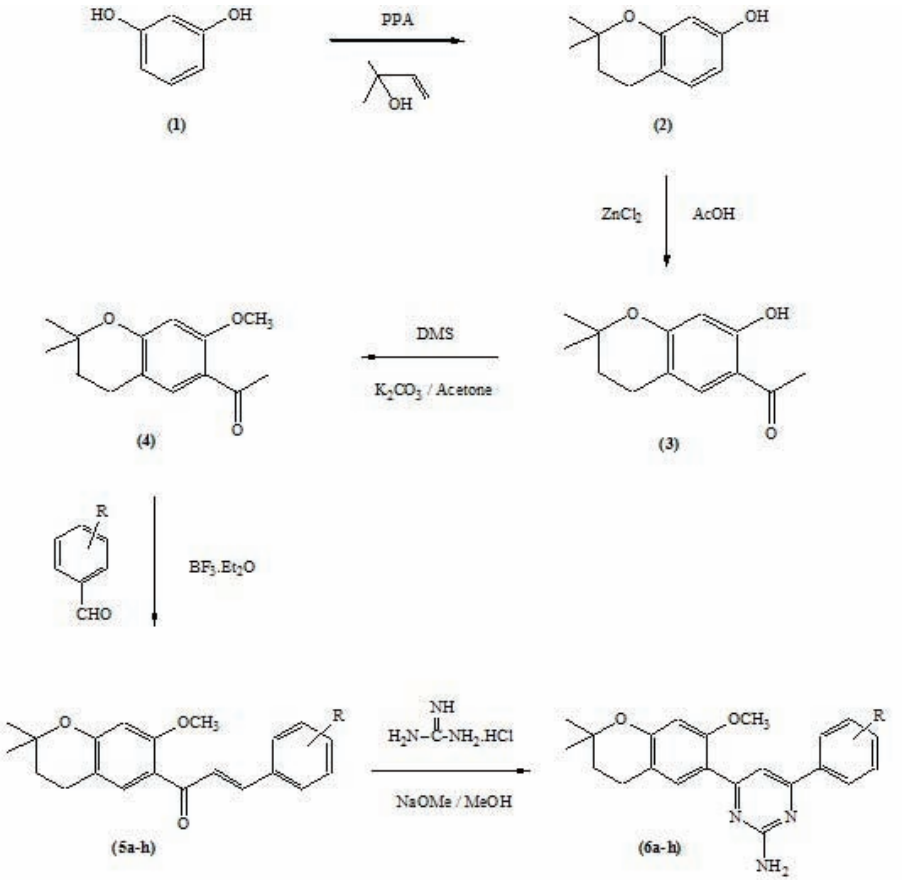
Materials, methods and instruments

All the chemicals and solvents used in this work were of analytical reagent grade (anhydrous) and purchased from Sigma–Aldrich. Melting points were determined using an electro-thermal apparatus in an open capillary tube and are uncorrected. The ^{1}H-NMR and ^{13}C--NMR spectra were recorded on a JEOL JNM Ex-90 instrument, at 90 and 22.5 MHz, respectively, using TMS as an internal reference in $CDCl_3$. The IR spectra were recorded in KBr discs on a Thermo Nicolet 6700 FTIR spectrophotometer. The HPLC was performed using a Shimadzu LC 6A instrument fitted with a Shimpack silica gel column using acetonitrile as the solvent. The mass spectra were obtained on a Varian Atlas CH-7 mass spectrometer. Elemental analyses were performed using an Elementar Vario EL elemental analyzer. Satisfactory C, H, N analyses were obtained for all the compounds.

The steps involved in the synthesis of the new series of 2-amino-4-benzopyran-6-yl pyrimidines from benzene-1,3-diol are depicted in Scheme 1.

*Synthesis of 3,4-dihydro-7-hydroxy-2,2-dimethyl-2*H*-benzopyran (**2**)*

To a solution of benzene-1,3-diol **1** (10 mmol) in dichloromethane (20 mL) was added polyphosphoric acid (0.1 eq.) followed by 2-methyl-3-buten-2-ol (5 mmol) and the mixture was stirred for 1 h at room temperature. The reaction mixture was heated at reflux for 4 h. After cooling, the mixture was neutralized with saturated $NaHCO_3$ and extracted with dichloromethane. The solvent was removed and the residue recrystallized from *n*-hexane to give a white crystalline solid in 68 % yield, which was characterized by its spectral data.[19]



R: a) H, b) 4-CH_3, c) 4-OCH_3, d) 2,5-OCH_3, e) $N(Me)_2$, f) 4-Cl, g) 4-NO_2, h) 4-CN

Scheme 1. The synthetic pathway to the new 2-amino benzopyrano pyrimidines **6a**–**h**.

*Synthesis of 6-acetyl-3,4-dihydro-7-hydroxy-2,2-dimethyl-2*H*-benzopyran (**3**)*

3,4-Dihydro-7-hydroxy-2,2-dimethyl-2*H*-benzopyran (**2**) (8 mmol) was acetylated using acetic acid (8 mmol) in the presence of freshly fused $ZnCl_2$ (8 mmol) at 140–150 °C under stirring for 15 min. After cooling to room temperature, HCl was added to the reaction mixture to break the zinc chloride complex and in 5 min precipitation commenced. The precipitate was washed with very dilute HCl to afford colorless needles in 54 % yield. The product was characterized by its spectral data.[19]

*Synthesis of 6-acetyl-3,4-dihydro-7-methoxy-2,2-dimethyl-2*H*-benzopyran (**4**)*

To a solution of 6-acetyl-3,4-dihydro-7-hydroxy-2,2-dimethyl-2*H*-benzopyran **3** (8 mmol) in acetone (20 mL), anhydrous K_2CO_3 (16 mmol) and dimethyl sulfate (8 mmol) were added and the mixture was stirred at room temperature for 2 h. The reaction mixture was poured into water, extracted with diethyl ether and dried over anhydrous Na_2SO_4. The residue was chromatographed using *n*-hexane and ethyl acetate (9:1) to give the compound as a colorless oil.

*General method for the synthesis of **5a–h***

To a stirred solution of 6-acetyl-3,4-dihydro-7-methoxy-2,2-dimethyl-2*H*-benzopyran **4** (6 mmol) and a substituted benzaldehyde (6 mmol) was added gradually $BF_3 \cdot Et_2O$ (3 mmol) at room temperature in dioxane. The solution was stirred for 2–3 h at room temperature. The reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled to room temperature and diluted with moist diethyl ether (50 mL), washed with water to discharge the color and the $BF_3 \cdot Et_2O$ complex. The extracted ethereal solution was dried over anhydrous Na_2SO_4, concentrated and recrystallized from dry ethanol to afford the appropriate pure chalcone.

*General procedure for the synthesis of **6a–h***

6-(Substituted cinnamoyl)-3,4-dihydro-7-methoxy-2,2-dimethyl-2*H*-benzopyran (5 mmol) and guanidine hydrochloride (5 mmol) were refluxed in presence of freshly prepared sodium methoxide (5 mmol) in methanol for 4–5 h. The solvent was evaporated and the residue was neutralized with 20 % HCl whereby a yellow solid separated out, which was filtered and crystallized from ethanol to give yellow crystals.

Antimicrobial activity

The *in vitro* antimicrobial activity determination was performed by the well-diffusion method[20] using 100 μg ml^{-1}. All the synthesized compounds (**6a**–**h**) were screened for antibacterial activity against 3 gram positive bacteria *Bacillus pumilus*, *Bacillus subtilis* and *Streptococcus faecalis*, 3 gram negative bacteria *Enterococcus faecalis*, *Escherichia coli* and *Proteus vulgaris* and 3 fungi *Pencillium expansum*, *Aspergillus niger* and *Candida albicans* using ampicillin and ketoconazole as standards in DMSO. DMSO showed no inhibition zone. Nutrient agar (NA) and potato dextrose agar (PDA) were used as the basal medium for test bacteria and fungi, respectively. Inhibition was recorded in millimeters by measuring the diameter of the inhibition zone at the end of 24 h for bacteria and 48 h for fungi with a Hi antibiotic zone scale at (35±2) °C. Each experiment was repeated twice and the average of the two determinations was recorded. The results are summarized in Tables I and II.

RESULTS AND DISCUSSIONS

The characterization data of all the synthesized compounds are given in the supplementary material to this paper.

Spectroscopy

According to the literature,[21] condensation of benzene-1,3-diol **1** with 2-methylbut-3-en-2-ol in presence of citric acid and phosphoric acid yielded 3,4-dihydro-7-hydroxy-2,2-dimethyl-*2H*-benzopyran in 55 and 43 % yield, respectively. In the present attempts to modify the reaction conditions, polyphosphoric acid was found to be a better condensing agent with 2-methyl-3-buten-2-ol leading to the cyclic benzopyran **2** in very good yield.

The preparation of 2-hydroxychalcones from 2-hydroxyacetophenones and benzaldehydes generally presents some difficulties because they always partially cyclize to flavanones during their synthesis, resulting in low yields.[22] Hence compound **3** was methylated to **4** using dimethyl sulfate to avoid byproducts and to obtain only chalcones in good yields. The sharp singlet at δ 3.71 in the ^{1}H-

-NMR, the peak at δ 53.6 in the ^{13}C-NMR and the disappearance of the band at 3433 cm^{-1} (OH) in the IR spectra confirmed the methoxy group of compound **4**. The synthesis of the title compounds was achieved *via* chalcone formation, which was generated *in situ* by the Claisen–Schmidt condensation of **4** with different aromatic aldehydes in presence of $BF_3 \cdot Et_2O$ within 2–3 h, without any side reactions.[23] Generally, in aqueous KOH- or NaOH-assisted synthesis of chalcones, the reaction times were much longer (1–2 days), with a high probability of side reactions, such as *via* the Cannizzaro reaction or aldol condensation. By using $BF_3 \cdot Et_2O$, the side reactions were reduced and chalcones were obtained exclusively, in good yields. The ^{1}H-NMR spectra of compounds **5a**–**h** show doublets of –CO–C**H**=CH–Ar and –CO–CH=C**H**–Ar at about δ 6.7 and 7.8 ppm, respectively, and the signals around δ 120 and 140 ppm in the ^{13}C-NMR spectra confirmed the presence of the chalcone moiety.

Finally, the target compounds were obtained by cyclization of **5a**–**h** in the presence of sodium methoxide in methanol with guanidine hydrochloride to give the products in good to excellent yields. The target compounds on purification and crystallization formed bright yellow crystalline solids of 99 % purity by HPLC. The synthesized 2-amino-4-benzopyran-6-ylpyrimidines (**6a–h**) exhibited characteristic primary amine bands at 3400–3100 cm^{-1} and did not show any absorption band in the region of 1700–1600 cm^{-1}, which indicates the absence of >C=O groups. The stretching peak of >C=N– appeared at 1600–1510 cm^{-1} and the absorption band at 855–800 cm^{-1} was characteristic of aromatic C–H bending vibrations. The ^{1}H-NMR spectrum showed the C_5-H proton as a singlet around δ 7.20–7.41 ppm and a broad signal around at δ 5.0 ppm due to the amino protons, which disappeared on D_2O exchange and the aromatic protons appeared between δ 7.2–7.9 ppm as a multiplet in all synthesized compounds as expected.[24] In the ^{13}C-NMR spectra of the pyrimidines, the C_2 carbon was the most deshielded carbon, appearing at δ 160 ppm, the C_4/C_6-carbons resonated at δ 157 ppm as expected.[25] The C_5 carbon resonated at δ 122 ppm nearer to the aromatic carbon shifts.

Antimicrobial screening

In the assay of the antimicrobial activity, all eight newly synthesized title compounds showed significant activity against *E. coli* with inhibition zones of 16 to 32 mm. Based on the inhibition zones shown by these compounds against all the tested bacterial strains, compound **6d** with methoxy groups at 2nd and 5th positions was found to be most effective against *E. coli* and *B. subtilis*, with activities nearly equal to those of the standard antibiotic ampicillin. This was explained as being due to the dimethoxy molecule being lipophilic[26] and good electron mobility in the aromatic ring may enhance the activity. It was demonstrated that increasing the number of methoxy groups on the phenyl ring or introducing an

electronic donating substituent on the phenyl ring resulted in increased antibacterial activity.

TABLE I. Inhibition zone (mean diameter of inhibition in mm) as a criterion of the antibacterial activities of the newly synthesized compounds

Compound	Gram-positive bacteria			Gram-negative bacteria		
	B. pumilus	*B. subtilis*	*S. faecalis*	*E. faecalis*	*E. coli*	*P. vulgaris*
6a	11	14	12	13	16	9
6b	12	15	16	14	18	11
6c	20	23	21	18	27	14
6d	27	29	23	20	32	22
6e	15	18	16	17	21	16
6f	8	14	–	14	24	12
6g	11	19	15	16	22	11
6h	10	11	13	–	19	10
Ampicillin	25	28	24	22	31	20

TABLE II. Inhibition zone (mean diameter of inhibition in mm) as a criterion of the antifungal activities of the newly synthesized compounds

Compound	Fungi		
	P. expansom	*A. niger*	*C. albicans*
6a	8	13	11
6b	–	16	11
6c	11	15	10
6d	17	16	–
6e	15	15	16
6f	21	24	18
6g	24	22	21
6h	18	17	15
Ketoconazole	22	23	19

From the screening results, it was observed that the presence of nitro and chloro groups at the phenyl ring in compounds **6f** and **6g** increased the antifungal activity against *A. niger* and their activity was equipotent to those of the standard ketoconazole. The other compounds, **6a**–**e** and **6h,** exhibited lower activities compared with those of the standard ketoconazole.

CONCLUSIONS

In conclusion, eight novel 2-amino-4-benzopyran-6-yl pyrimidines (**6a**–**h**) were synthesized from benzene-1,3-diol and were subjected to *in vitro* antimicrobial activity screening against various pathogenic bacteria and fungi. The screening results indicated that all the compounds exhibited moderate activity against bacteria and fungi, except the **6d, 6f** and **6g** compounds. These differences in activity depended on the substitution of different reactive groups on the pyrimidine moiety. Further studies to explore the structure–activity relationship

by structural modifications of the final investigated compounds to improve their biological activity are in progress.

SUPPLEMENTARY MATERIAL

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

ИЗВОД

СИНТЕЗА И КАРАКТЕРИЗАЦИЈА НОВИХ 2-АМИНО-4-(3,4-ДИХИДРО-2,2-ДИМЕТИЛ-7-МЕТОКСИ-2*H*-БЕНЗОПИРАН-6-ИЛ)-6-(СУПСТИТУИСАНИ ФЕНИЛ)ПИРИМИДИНА И ИСПИТИВАЊЕ ЊИХОВЕ БИОЛОШКЕ АКТИВНОСТИ

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Синтетисана је нова серија од осам деривата 2-амино-4-(3,4-дихидро-2,2-диметил-7-метокси-2*H*-бензопиран-6-ил)-6(супституисани фенил)пиримидина, употребом бензен 1,3-диола као полазног једињења. Структура изолованих производа (**6a–h**) утврђена је спектроскопским техникама ^{1}H-NMR, ^{13}C-NMR и FT-IR и елементалном анализом. Испитана је биолошка активност синтетисаних једињења према грам-позитивним (*Bacillus pumilus*, *Bacillus subtilis* и *Streptococcus faecalis*) и грам-негативним (*Enterococcus faecalis*, *Escherichia coli* и *Proteus vulgaris)* бактеријама и гљивама *(Pencillium expansum*, *Aspergillus niger* и *Candida albicans*). Већина деривата показује умерену до високу антибактеријску и фунгицидну активност, са зонама инхибиције између 8 и 30 mm.

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