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Synthesis and Self-Assembling Properties of β-D-Glucuronosyl-5-acetyl-7-[(1-acetyl-5-aryl-4,5-dihydro-1*H*-pyrazol-3-yl)-amino]-1,2-benzisoxazole-3-carboxylates

Rajendra K. Wanare

Department of Chemistry, Jawaharlal Nehru Arts, Commerce and Science College, Wadi, Nagpur-440023, RTM, Nagpur University, Nagpur (MS) India
Corresponding author. Mob.: +91 9730039437; fax: +91 7104-220963.
E-mail address: rajwanare@rediffmail.com
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Abstract: Condensation of *N*-(5-acetyl-3-methyl-1,2-benzisoxazol-7-yl)-3-arylprop-2-enamides (**1a-k**) with hydrazine hydrate and acetic acid yielded 1-{3-Methyl-7-[(1-acetyl-5-aryl-4,5-dihydro-1*H*-pyrazol-3-yl)-amino]-1,2-benzisoxazol-5-yl}-ethan-1-ones (**2a-k**).5-acetyl-7-[(1-acetyl-5-aryl-4,5-dihydro-1*H*-pyrazol-3-yl)-amino]-1,2-benzisoxazole-3-carboxylic acids (**3a-k**) prepared by the oxidation of (**2a-k**) with $KMnO_4$.β-D-Glucuronosyl-5-acetyl-7-[(1-acetyl-5-aryl-4,5-dihydro-1*H*-pyrazol-3-yl)-amino]-1,2-benzisoxazole-3-carboxylates (**4a-k**) prepared by the glucuronidation of (**3a-k**)with free D-gluconic. The structure of compoundswascharacterized on the basis of their instrumental analysis FT-IR, ^{1}H-NMR, FAB-MS, elemental analysis and chemical properties. Some compounds showed significant antibacterial activity against *E. coli* and *S. aureus* and moderate to antifungal activity against *A. niger* and *C. albicans*.

Keywords: β-D-Glucuronides, 1,2-Benzisoxazoles, Pyrazoles, Chalcones, Antibacterial activity.

INTRODUCTION

Glucuronidation is a major detoxification pathway in mammalian liver, where UDP-glucuronosyltransferases catalytically conjugate hydrophobic xenobiotics and endobiotic to glucuronic acid, thereby increasing their solubility. Studies have shown that the parent compound is metabolized into glucuronidated metabolites in rats after oral genistein administration. Drug metabolism is closely related to its pharmacological activity and are polar, chemically reactive and generating increasing interest as potential mediator of hypersensitivity reaction which shows profound effect on drug metabolism. Biotransformation is largely catalysed in the liver and intestine which are rich in drug-metabolizing enzymes[1-6].1,2-benzisoxazoles are biologically active molecules with potential applications in drug design. 1,2-Benzisoxazole bears a close structural resemblance to indole

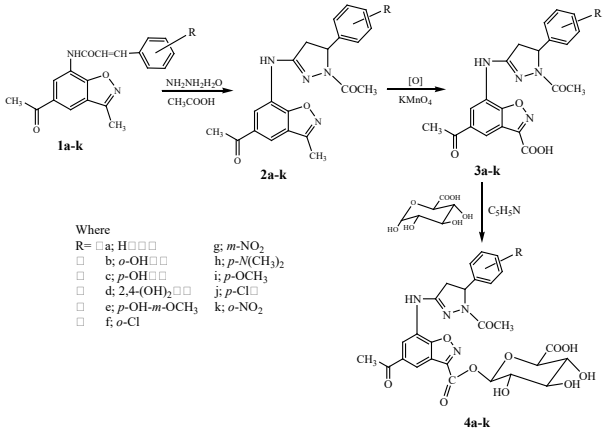
which shows antipsychotic, antitumor, antithrombotic, anti-inflammatory, analgesic, sedative and tuberculostearic agents. Various 1,2-benzisoxazole derivatives have been found to possess antiepileptic, neurotoxic, antidepressant, hypertensive, anticonvulsant and antimicrobial activities [7-9]. Pyrazole and its derivatives are considered a pharmacologically important active scaffold that possesses almost all types of pharmacological activities like remarkable antileishmanial agent, anticancer, antidepressant, antiviral, antioxidant, anti-inflammatory, anti-tuberculosis, antibacterial, as well as antifungal agents. This moiety can be traced in a number of well-established drugs belonging to different categories with diverse therapeutic activities [10-15]. In recent years chalcones have attracted much attention due to their diverse properties and wide spectrum of biological and pharmacological activities like germicidal, anti-tuberculous, antifungal, bacteriostatic, anticancer, antimalarial, antitumor and antiparasitic properties. The potential properties of natural occurring chalcones like anti-inflammatory, antioxidant, anticancer, antiparasitic and antimicrobial, and their unique chemical structural features inspired the synthesis of numerous chalcone derivatives. The compounds of the chalconesseries also show profound effects on the cardiovascular, cerebrovascular and neuromuscular system including the vital organs of the experimental animals[16-21].

RESULT AND DISCUSSION

In order to further understand the structural influence on molecular self-assembling and gelation properties, in this study we inserted biological applications of β-D-glucuronidesobtained in **Scheme**, herein promoted us to prepare new class of pyrazoles and β-D-glucuronides. 1-{3-Methyl-7-[(1-acetyl-5-aryl-4,5-dihydro-1*H*-pyrazol-3-yl)-amino]-1,2-benzisoxazol-5-yl}-ethan-1-ones (**2a-k**) were prepared by condensing*N*-(5-acetyl-3-methyl-1,2-benzisoxazol-7-yl)-3-arylprop-2-enamides (**1a-k**) with hydrazine hydrate and acetic acid. Similarly, 5-acetyl-7-[(1-acetyl-5-aryl-4,5-dihydro-1*H*-pyrazol-3-yl)-amino]-1,2-benzisoxazole-3-carboxylic acids (**3a-k**) were synthesized by the oxidation of (**2a-k**) with $KMnO_4$. Glucuronidation of (**3a-k**) with D-gluconic acid and pyridine yielded β-D-glucuronosyl-5-acetyl-7-[(1-acetyl-5-aryl-4,5-dihydro-1*H*-pyrazol-3-yl)-amino]-1,2-benzisoxazole-3-carboxylates (**4a-k**).Their structure was assigned with elemental analysis, IR, ^{1}H NMR and FAB-Mass spectral analysis.

MATERIALS AND METHODS

Melting points were determined in open capillary tubes and are uncorrected. IR spectra were recorded as KBr pallets on Shimadzu-810 IA and Perkin Elmer FT-IR spectrometer (υ_{max} in cm^{-1}). ^{1}H NMR spectra were recorded on Bruker AC-300F (300MHz) instrument with TMS as internal standard. Mass spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer using *m*-nitrobenzyl alcohol (NBA) matrix.All other reagents were freshly distilled.



Scheme

1-{3-Methyl-7-[(1-acetyl-5-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)-amino]-1,2-benzisoxazol-5-yl}-ethan-1-one (2a)

Compounds (**1a-k**) have been previously described[22]. Cycloaddition of *N*-(5-acetyl-3-methyl-1,2-benzisoxazol-7-yl)-3-phenylprop-2-enamide **1a** (0.01 mole, 3.20g) with hydrazine hydrate (0.01 mole) and few drops of acetic acid yielded 1-{3-Methyl-7-[(1-acetyl-5-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)-amino]-1,2-benzisoxazol-5-yl}-ethan-1-one **2a**.Reaction mixture was cooled and poured onto ice. The obtained solid was filtered, wash with water and crystallized from appropriate solvent (1.9g, 59.3%), m.p. 105^0C. IR (KBr): 1730 and 1665 (two C=O ketones), 1480 (N-N of pyrazole), 1572 (C=N), 3410 (N-H), 3009 (Ar-H), 2932 (methyl C-H) cm^{-1}; ^{1}H NMR ($CDCl_3$): signal at δ2.6 (s, 3H, CH_3), 7.2-8.6 (m, aromatic protons), 2.61(m, 2H, pyrazole CH_2), 5.76 (t, Ar-CH), 3.42 (s, $COCH_3$), 8.5 (s, 1H, NH); FAB-MS: M^+ 376, m/z 334, m/z 258, m/z 190and m/z 175.
Following the above procedure 1-{3-Methyl-7-[(1-acetyl-5-ary-4,5-dihydro-1*H*-pyrazol-3-yl)-amino]-1,2-benzisoxazol-5-yl}-ethan-1-ones (**2a-k**) were prepared and compounds gave satisfactory C, H, and N analysis (**Table 1**).

5-Acetyl-7-[(1-acetyl-5-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)-amino]-1,2-benzisoxazole-3-carboxylic acid (3a)

Mixture of 1-{3-Methyl-7-[(1-acetyl-5-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)-amino]-1,2-benzisoxazol-5-yl}-ethan-1-one **2a** (0.01mol, 3.76g), sodium carbonate (1.5g), $KMnO_4$ (1.5g) and water (100ml) was refluxed under water bath for 4hrs, until the purple colour has disappeared. Acidified with dil. H_2SO_4 and excess manganese dioxide was removed by sodium metabisulphite (0.1g), filtered, washed and crystallized with water (2.1g, 55.8%), m.p. 101^0C. IR (KBr): 3463 (OH peak), 1650 and 1664 (two C=O ketones), 1762 (carboxylic =C=O), 1484 (N-N of pyrazole), 1362 (C=N ter. amine), 3410 (N-H); ^{1}H-NMR signal at δ10.3 (s, COOH), 6.2-7.9 (m, aromatic protons), 2.61 (m, 2H, pyrazole CH_2), 8.3 (s, 1H, NH); FAB-MS: M^+406, m/z 364, m/z 330, m/z 288, m/z 220, m/z 205 and m/z 176.

5-Acetyl-7-[(1-acetyl-5-aryl-4,5-dihydro-1*H*-pyrazol-3-yl)-amino]-1,2-benzoxazole-3-carboxylic acids (**3a-k**) were synthesized using same procedure.

β-D-Glucuronosyl-5-acetyl-7-[(1-acetyl-5-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)-amino]-1,2-benzisoxazole-3-carboxylates (4a)

Reaction of **3a**(0.01 mol, 4.06g) with glucuronic acid (1.94g) anddry pyridine (5ml) at 0^0C and it was left for 18hrs. White product poured onto ice, filtered and washed with cold water (2.20g, 54.1%). FAB-MS: M^+ 582, the base peak appearing at m/z 406 (due toloss of glucuronic acid moiety), m/z 376, m/z334, m/z 300, m/z 258, m/z 190 and m/z 175.

Applying the above procedure, β-D-Glucuronosyl-5-acetyl-7-[(1-acetyl-5-aryl-4,5-dihydro-1*H*-pyrazol-3-yl)-amino]-1,2-benzisoxazole-3-carboxylates (**4a-k**) werepreparedstarting from appropriate carboxylicacids (**3a-k**). Compounds gave satisfactory C, H, and Nanalysis (**Table 2**).

ANTIBACTERIAL ACTIVITY

New compounds (**4a-k**) were tested for their antibacterial activities by using the cupplate method against *S. aureus*and *E. coli* atconcentration of 150µg/mL in DMF. The screening results showed moderate to excellent activity against both organisms. The antifungal activity of synthesizedcompounds was evaluated by the using above same procedure against *Aspergillus niger* and *Candida albicans* at a concentration 100µm/mL in DMF. The screening results showed moderate to excellent activity against both organisms (**Table 3**).

CONCLUSION

Newly synthesized β-D-Glucuronosyl-5-acetyl-7-[(1-acetyl-5-aryl-4,5-dihydro-1*H*-pyrazol-3-yl)-amino]-1,2-benzisoxazole-3-carboxylates (**4a-k**)compounds were evaluated for in vitro antibacterial activity against *E. coli* and *S. aureus*strains as well as for antifungal activity against *A. niger* and*C. albicans* strains using cup-plate technique. Manycompounds gave excellent results against bacterial and fungal strain. Compounds are confirmed by FT-IR, ^{1}H-NMR, FAB-MS, optical activity and elemental analysis.

Table 1. Characterization data of 1-{3-Methyl-7-[(1-acetyl-5-aryl-4,5-dihydro-1*H*-pyrazol-3-yl)-amino]-1,2-benzisoxazol-5-yl}-ethan-1-one (**2a-k**)

Comp	R	Molecular Formula	Mol. Wt.	M.P. (^{0}C)	R_f Value	Calculated (Found) %		
						C	H	N
2a	H	$C_{21}H_{20}N_4O_3$	376.4	116	0.24	67.01 (67.00)	5.36 (5.32)	14.88 (14.87)
2b	*o*-OH	$C_{21}H_{20}N_4O_4$	392.4	109	0.30	64.28 (64.27)	5.14 (5.12)	14.28 (14.26)
2c	*p*-OH	$C_{21}H_{20}N_4O_4$	392.4	111	0.32	64.28 (64.26)	5.14 (5.14)	14.28 (14.26)
2d	2,4-$(OH)_2$	$C_{21}H_{20}N_4O_5$	408.4	101	0.28	61.76 (61.74)	4.94 (4.93)	13.72 (13.71)
2e	*p*-OH-*m*-OCH_3	$C_{22}H_{22}N_4O_5$	422.4	107	0.31	62.55 (62.56)	5.25 (5.23)	13.26 (13.20)
2f	*o*-Cl	$C_{21}H_{19}ClN_4O_3$	410.8	118	0.26	61.39 (61.38)	4.66 (4.65)	13.64 (13.59)
2g	*m*-NO_2	$C_{21}H_{19}N_5O_5$	421.4	119	0.30	59.85 (59.83)	4.54 (4.51)	16.62 (16.59)
2h	*p*-*N*$(CH_3)_2$	$C_{23}H_{25}N_5O_3$	419.4	103	0.24	65.85 (65.85)	6.01 (6.00)	16.70 (16.70)
2i	*p*-OCH_3	$C_{22}H_{22}N_4O_4$	406.4	104	0.31	65.01 (65.00)	5.46 (5.44)	13.78 (13.75)
2j	*p*-Cl	$C_{21}H_{19}ClN_4O_3$	410.8	113	0.24	61.39 (61.37)	4.66 (4.63)	13.64 (13.58)
2k	*o*-NO_2	$C_{21}H_{19}N_5O_5$	421.4	109	0.27	59.85 (59.83)	4.54 (4.52)	16.62 (16.63)

Table 2. Characterization data of β-D-Glucuronosyl-5-acetyl-7-[(1-acetyl-5-aryl-4,5-dihydro-1*H*-pyrazol-3-yl)-amino]-1,2-benzisoxazole-3-carboxylates (**4a-k**)

Comp	R	Molecular Formula	Mol. Wt.	$[\alpha]_D^{25}$ (^{0})	R_f Value	Calculated (Found) %		
						C	H	N
4a	H	$C_{27}H_{26}N_4O_{11}$	582.5	+44.1	0.30	55.67 (55.66)	4.50 (4.48)	9.62 (9.60)
4b	*o*-OH	$C_{27}H_{26}N_4O_{12}$	598.5	+41.4	0.28	54.18 (54.14)	4.38 (4.35)	9.36 (9.34)
4c	*p*-OH	$C_{27}H_{26}N_4O_{12}$	598.5	+38.4	0.32	54.18 (54.17)	4.38 (4.36)	9.36 (9.32)
4d	2,4-$(OH)_2$	$C_{27}H_{26}N_4O_{13}$	614.5	+41.1	0.28	52.77 (52.75)	4.26 (4.25)	9.12 (9.11)
4e	*p*-OH-*m*-OCH_3	$C_{28}H_{28}N_4O_{13}$	628.5	+42.5	0.30	53.50 (53.47)	4.49 (4.39)	8.91 (8.91)
4f	*o*-Cl	$C_{27}H_{25}ClN_4O_{11}$	616.9	+30.7	0.31	52.56 (52.40)	4.08 (4.02)	9.08 (9.10)
4g	*m*-NO_2	$C_{27}H_{25}N_5O_{13}$	627.5	+41.8	0.32	51.68 (51.68)	4.02 (4.01)	11.16 (11.14)
4h	*p*-*N*$(CH_3)_2$	$C_{29}H_{31}N_5O_{11}$	625.5	+44.1	0.27	55.68 (55.64)	4.99 (4.97)	11.19 (11.15)
4i	*p*-OCH_3	$C_{28}H_{28}N_4O_{12}$	612.5	+38.8	0.30	54.90 (54.89)	4.61 (4.60)	9.15 (9.15)
4j	*p*-Cl	$C_{27}H_{25}ClN_4O_{11}$	616.9	+38.3	0.24	52.56 (52.53)	4.08 (4.07)	9.08 (9.02)
4k	*o*-NO_2	$C_{27}H_{25}N_5O_{13}$	627.5	+40.1	0.22	51.68 (51.60)	4.02 (3.98)	11.16 (11.15)

Table 3. Data for in vitro antibacterial and antifungal activities of compounds (**4a-k**)

Products	Diameter of Inhibition Zone (in mm) Against			
	Bacterial Strains		Fungal Strain	
	E. Coli	*S. aureus*	*A. niger*	*C. albicans*
4a	--	15	22	24
4b	16	11	--	27
4c	15	14	21	18
4d	13	--	28	20
4e	14	16	--	22
4f	10	12	19	--
4g	16	15	24	18
4h	12	--	23	20
4i	--	14	18	22
4j	16	10	20	--
4k	14	13	18	28

-- = No inhibition of growth. Diameter of zone of inhibition from 13-16 (in mm) shows

excellent activity and that of 9-12 (in mm) exhibit moderate activity for bacterial strains. Diameter of zone of inhibition from 22-28 (in mm) shows excellent activity, that of 15-21 (in mm) exhibits moderate activity and that of 11-14 (in mm) shows poor activity for fungal strains. Norfloxacin 100µg/mL used as standard against *E. coli* and *S. aureus*diameter of zone of inhibition is 20. Griseofulvin 100µm/mL used as standard against *A. niger* and *C. albicans* diameter of zone of inhibition is 32.

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