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A SIMPLE AND HIGHLY EFFICIENT PROCEDURE FOR SYNTHESIS OF PYRAZOLES AND *O*-GLUCOSIDES

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Abstract: Cyclization of 1-(3'-Methyl benzisoxazol-5'-yl)-3-phenyl prop-2-en-1-one **1a** with hydrazine hydrate to produce 3-methyl-5-(3-phenyl-1H-pyrazol-5-yl)benzo[d]isoxazole **2a**. Oxidation of compound **2a** with $KMnO_4$ furnishes 5-(3'-Phenyl-1H-pyrazol-5'-yl)-1,2-benzisoxazole-3-carboxylic acid **3a**. Glucosylation of 5-(3'-Phenyl-1H-pyrazol-5'-yl)-1,2-benzisoxazole-3-carboxylic acid **3a** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (TAGBr) **4** to afford tetra-acetyl derivative **5a** followed by deacetylation to give (2S,3S,4S,5S)-tetrahydro-3,4,5-trihydroxy-6-(hydroxymethyl)-2H-pyran-2-yl-5-(3-phenyl-1H-pyrazol-5-yl)benzo[d]isoxazole-3-carboxylate **6a**. The structures of the products have been assigned on the basis of FT-IR spectra, ^{1}H NMR, ^{13}C NMR, FAB-MS, optical activity and elemental analysis. All the synthesized compounds were evaluated their antibacterial and antifungal activities by cup-plate method. The present approach offers several advantages such as shorter reaction times, cleaner reactions, good yields, inexpensive reagent and mild reaction conditions.

Keywords: Chalcones, Pyrazoles, Carboxylic acids, TAGBr, *O*-Glucosides.

1. Introduction

Glucosylation improve the solubility of various drugs without affecting their activities and attaching of the glucosidic moiety into the molecule increases its hydrophilicity than the respective aglycone moiety and it can improve the drug targeting to the cells due to their solubility in the membrane components. Glucosylation reaction is the key reaction for the synthesis of many carbohydrate based biomolecules, oligosaccharides, complex carbohydrate conjugates and many complex glucosides. Glucosides are the acetals of alcohols or phenols and they are widely distributed in nature in plants and animals. Glucosylation reaction is the key reaction for the synthesis of many carbohydrate based biomolecules. In glycosides, the non-carbohydrate moiety attached to the sugar molecule is known as aglycone, hence glycosides composed of a sugar residue attached to aglycone moiety. Glucosides are normally water soluble and optically active compounds and major role is that they act as main carrier of the aglycone

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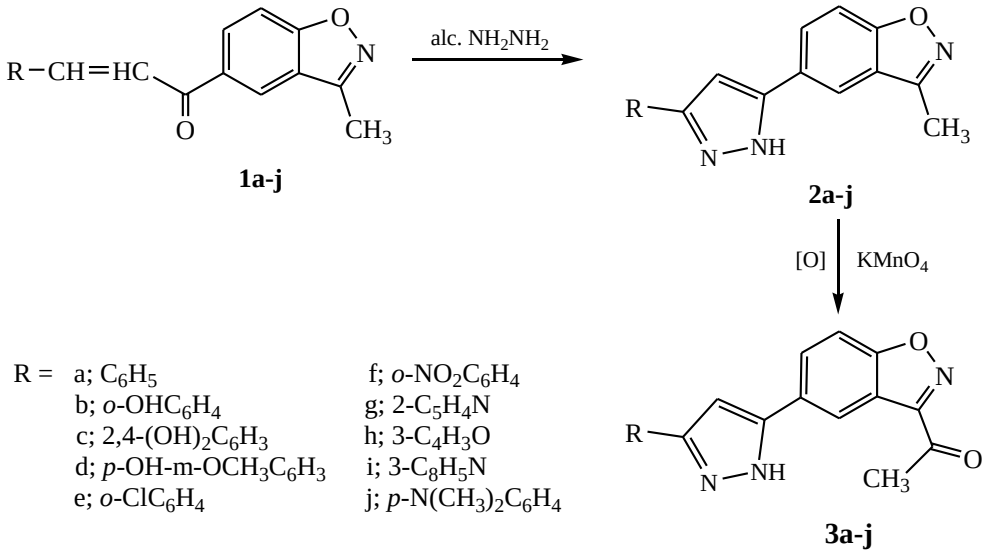
moiety. These glucosides are not responsible for any pharmacological activities, they directly targets to the enzyme active site to the aglycone portion. The carbohydrate moiety enhances the water and lipid solubility of the pharmacophoric group and major active molecule is the aglycone which is responsible for its biological activities[1-3]. Continuing our studies about heterocyclic compounds and their glucosides, herein we want to describe the synthesis of Pyrazoles, Carboxylic acids and *O*-Glucosides. The activities of pyrazoles include main topics like remarkable antimicrobial, antioxidant, fungicidal, bactericidal, bacteriostatic, sedative, antipyretic, analgesic, anti-inflammatory, muscle relaxant, hypoglycemic and sex stimulating agents. Many pyrazoles are used for the treatment of thyroid and leukemia having possessed wide range of pharmacological activities like anti-invasive, anti-depressant etc[4–10].



Scheme 1. Synthesis of Pyrazoles and Carboxylic acids

2. Experimental

All chemicals and reagents of great quality were purchased commercially. All purchased starting materials were used without further purification. The determined melting points are uncorrected. NMR spectra were recorded on 300MHz instruments. The mass spectra were recorded under ESI mode, on Thermo Finnigan (Model-LCQ Advantage MAX) mass spectrometer. Absorption peaks of functional groups were observed by Fourier-transform Infrared (FT-IR) on Shimadzu FT-IR spectrometer DRS-84000 using KBr pellets.

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2.1. Chemistry

2.1.1. *General Procedure for Synthesis of **3a-j**:*

A mixture of 1-(3'-methyl benzisoxazol-5'-yl)-3-phenyl prop-2-en-1-one **1a** (2.6g, 0.01 mol), hydrazine hydrate (0.5 ml), ethyl alcohol (15 ml) and KOH (0.6g) was refluxed on water bath for 5 hrs. The progress of reaction was monitored by Thin Layer Chromatography (TLC). After completion of reaction, the reaction mixture was cooled to room temperature, diluted with acetic acid and poured on 50 ml crush ice water. The separated solid was filtered, washed with water, dried and crystallized from the appropriate solvent to give the corresponding 3-methyl-5-(3-phenyl-1H-pyrazol-5-yl)benzo[d]isoxazole **2a**. In the same ways, pyrazole derivatives were prepared and products gave satisfactory C, H and N analysis (**Table 1**).

3-methyl-5-(3-phenyl-1H-pyrazol-5-yl)benzo[d]isoxazole (**2a**): Yield 68.5%, m.p. 105^0C, FT-IR (KBr): 3305, 1715, 1562, 903 cm^{-1}, ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.81 (C-H pyrazole), 13.7 (s, N-H), 2.35 (CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$+DMSO-d_6) 121-145 (C-2, C-3), 189 (C-1), 155 (s, benzisoxazole), 99 (s, pyrazole), 15.9 (s, CH_3), 127.5-133.1 (m, benzene); Calculated for $C_{17}H_{13}N_3O$, MS (m/z): 275 found 275.3 (M^+).

2-(5-(3-methylbenzo[d]isoxazol-5-yl)-1H-pyrazol-3-yl)phenol (**2b**): Yield 53.75%, m.p. 120^0C, FT-IR (KBr): 3520, 3304, 1730, 1561, 907 cm^{-1}, ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.48, 7.32 (benzene), 6.81 (C-H pyrazole), 13.7 (s, N-H), 2.34 (CH_3), 5.0 (aromatic, C-OH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$+DMSO-d_6) 120-148 (C-2, C-3), 128 (C-1), 127-155 (s, benzisoxazole), 148 (s, pyrazole), 15.9 (s, CH_3), 99.7-130 (m, benzene); Calculated for $C_{17}H_{13}N_3O_2$, MS (m/z): 291 found 292 (M^+).

5-(3-(2-chlorophenyl)-1H-pyrazol-5-yl)-3-methylbenzo[d]isoxazole (**2e**): Yield 67.5%, m.p. 99^0C, FT-IR (KBr): 3304, 1730, 1561, 907, 710 cm^{-1}, ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.33, 7.48 (benzene), 6.81 (C-H, pyrazole), 13.7 (s, N-H, pyrazole), 2.35 (CH_3), 0.06 (C-Cl), 7.33-7.42 (benzene) ppm; ^{13}C NMR (100 MHz, $CDCl_3$+DMSO-d_6) 122-148 (C-2, C-3), 129 (C-1, benzene), 127-155 (s, benzisoxazole), 99.7-148.8 (s, pyrazole), 15.9 (s, CH_3), 127.4-130 (m, benzene); Calculated for $C_{17}H_{12}ClN_3O$, MS (m/z): 309 found 309 (M^+).

A mixture of 3-methyl-5-(3-phenyl-1H-pyrazol-5-yl)benzo[d]isoxazole **2a** (2.7g, 0.01 mol), $KMnO_4$ (1.5g), sodium carbonate (1.2g) and H_2O (100 ml) was refluxed under water bath for 4 hrs until the color of permanganate disappeared and acidified with dilute H_2SO_4. The excess manganese dioxide was removed by adding sodium metabisulphite (0.1g). The separated solid was filtered, washed with water, dried and crystallized from the appropriate solvent to give the corresponding carboxylic acid i.e. 5-(3'-Phenyl-1H-pyrazol-5'-yl)-1,2-benzisoxazole-3-

carboxylic acid **3a**. Similarly, other carboxylic acid derivatives were prepared and products gave satisfactory C, H and N analysis.

5-(3'-Phenyl-1H-pyrazol-5'-yl)-1,2-benzisoxazole-3-carboxylic acid (**3a**): Yield 50.9%, m.p. 111^0C, FT-IR (KBr): 3094, 1688, 1591, 903 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.81 (C-H pyrazole), 13.7 (N-H), 2.36 (CH_3), 10.7 (COOH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$+DMSO-d_6) 126-147 (benzisoxazole), 99.7 and 148 (C-H, pyrazole), 167 (s, COOH), 127-133.1 (benzene); Calculated for $C_{17}H_{11}N_3O_3$, MS (m/z): 305 found 305.1 (M^+).

1-(5-(3-(2,4-dihydroxyphenyl)-1H-pyrazol-5-yl)benzo[d]isoxazol-3-yl)ethanone (**3c**): Yield 42.9%, m.p. 170^0C, FT-IR (KBr): 3540, 3580, 3505, 3093, 1706, 1590, 905 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.81 (C-H pyrazole), 13.7 (N-H), 2.55 (CH_3), 6.26-7.14 (C-H, benzene), 10.8 (s, COOH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$+DMSO-d_6) 127-146 (benzisoxazole), 99.6 and 147 (C-H, pyrazole), 168 (s, COOH), 127-133.3 (benzene); Calculated for $C_{17}H_{11}N_3O_5$, MS (m/z): 337 found 337.3 (M^+).

1-(5-(3-(2-nitrophenyl)-1H-pyrazol-5-yl)benzo[d]isoxazol-3-yl)ethanone (**3f**): Yield 50.8%, m.p. 161^0C, FT-IR (KBr): 1570~1490,1390~1300, 1530, 1310, 3090, 1705, 910 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.81 (C-H pyrazole), 13.7 (N-H), 2.55 (CH_3), 7.74-8.25 (C-H, benzene), 10.9 (COOH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$+DMSO-d_6) 125-147 (benzisoxazole), 99.1 and 146 (C-H, pyrazole), 165 (COOH), 125-133.8 (benzene); Calculated for $C_{17}H_{10}N_4O_5$, MS (m/z): 350 found 350.5 (M^+).

2.1.2. *General Procedure for Synthesis of **6a-j**:*

Glucosylation. To a solution of 5-(3'-phenyl-1H-pyrazol-5' -yl)-1,2-benzisoxazole-3-carboxylic acid 3a (0.01 mol, 3.05g) and 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (TAGBr) 4 in CH_2Cl_2 was added tetrabutyl ammonium bromide (0.32g) with stirring at 5^0C. Sodium hydroxide (10%, 10 ml) was added to it drop wise over a period of 30 minutes and the reaction mixture further stirred for 24 hrs. Separated tetra-acetyl derivative 3-(2,3,4,6-tetra-O-acetyl-4'-O-β-D-glucosidoxyphenyl)-5-(3'-phenyl-1H-pyrazol-5'-yl)-1,2-benzisoxazole 5a was washed with H_2O, 5% dilute $NaHCO_3$ and again with water.

Deacetylation. To a solution of tetra-acetyl derivative 5a in absolute methanol (25 ml) was added sodium methoxide solution (0.5%, 1.5 ml) and kept at room temperature for about 1hr. The reaction mixture was neutralized with ion exchange resin (Amberlite IR 120) and filter. A semisolid mass was purified on column of silica gel and crystallized from suitable solvent to produce brown syrupy product β-D-glucopyranosyl-5-(3'-phenyl-1H-pyrazol-5'-yl)-1,2-benzisoxazole-3-carboxylate 6a. Similarly, other *O*-glucoside derivatives were prepared and products gave satisfactory C, H and N analysis (**Table 2**).

(2S,3S,4S,5S)-tetrahydro-3,4,5-trihydroxy-6-(hydroxymethyl)-2H-pyran-2-yl-5-(3-phenyl-1H-pyrazol-5-yl)benzo[d]isoxazole-3-carboxylate (**6a**): Yield 59.0%, $[\alpha]_D^{25}$ +44.7; FT-IR (KBr): 3296, 3000, 1688, 1591, 1489, 1235, 1092 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 6.2-7.5 (m, 9H aromatic), 4.7-5.8 (m, 4H sugar) ppm. The PMR spectrum displayed no signals of acetyl protons, signal due to protons of the carbohydrate hydroxyl group were not observed in the spectrum because of the fast exchange of all non-hydrogen bonded –COOH groups; Calculated for $C_{23}H_{21}N_3O_8$, MS (m/z): 467 found 467.2 (M^+).

3. Result and Discussion

3.1. Chemistry

A series of chalcone derivatives were prepared by condensation reaction of different aromatic aldehydes with ketone *via* Claisen-Schmidt condensation as reported in similar earlier work[11]. The reaction of 1-(3'-methyl benzisoxazol-5'-yl)-3-phenyl prop-2-en-1-one **1a** with $NH_2NH_2.H_2O$ and alcoholic KOH for 5hrs, cyclization occurred to form 3-methyl-5-(3'-phenyl-1*H*-pyrazol-5'-yl)-1,2-benzisoxazole **2a**. Oxidation of compound **2a** with oxidizing agent $KMnO_4$ to afford 5-(3'-phenyl-1*H*-pyrazol-5'-yl)-1,2-benzisoxazole-3-carboxylic acid **3a**. Pronounced biological and pharmacological importance of *O*-glucosides, (2S,3S,4S,5S)-tetrahydro-3,4,5-trihydroxy-6-(hydroxymethyl)-2H-pyran-2-yl-5-(3-phenyl-1H-pyrazol-5-yl)benzo[d]isoxazole-3-carboxylate **6a** have been prepared by the glucosylation of 5-(3'-phenyl-1*H*-pyrazol-5'-yl)-1,2-benzisoxazole-3-carboxylic acid **3a** and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (TAGBr) **4** in CH_2Cl_2 in the presence of tetrabutyl ammonium bromide (PTC) and followed by deacetylation of 3-(2,3,4,6-tetra-*O*-acetyl-4'-*O*-β-D-glucosidoxyphenyl)-5-(3'-phenyl-1*H*-pyrazol-5'-yl)-1,2-benzisoxazole **5a**. Based on the above method, the synthesis of pyrazoles, carboxylic acids and *O*-glucoside derivatives are discussed in details (**Scheme 1, 2 and Table 1, 2**). Advantage of this method is that the reagent is non toxic, cheaply available and stable under the reaction conditions.

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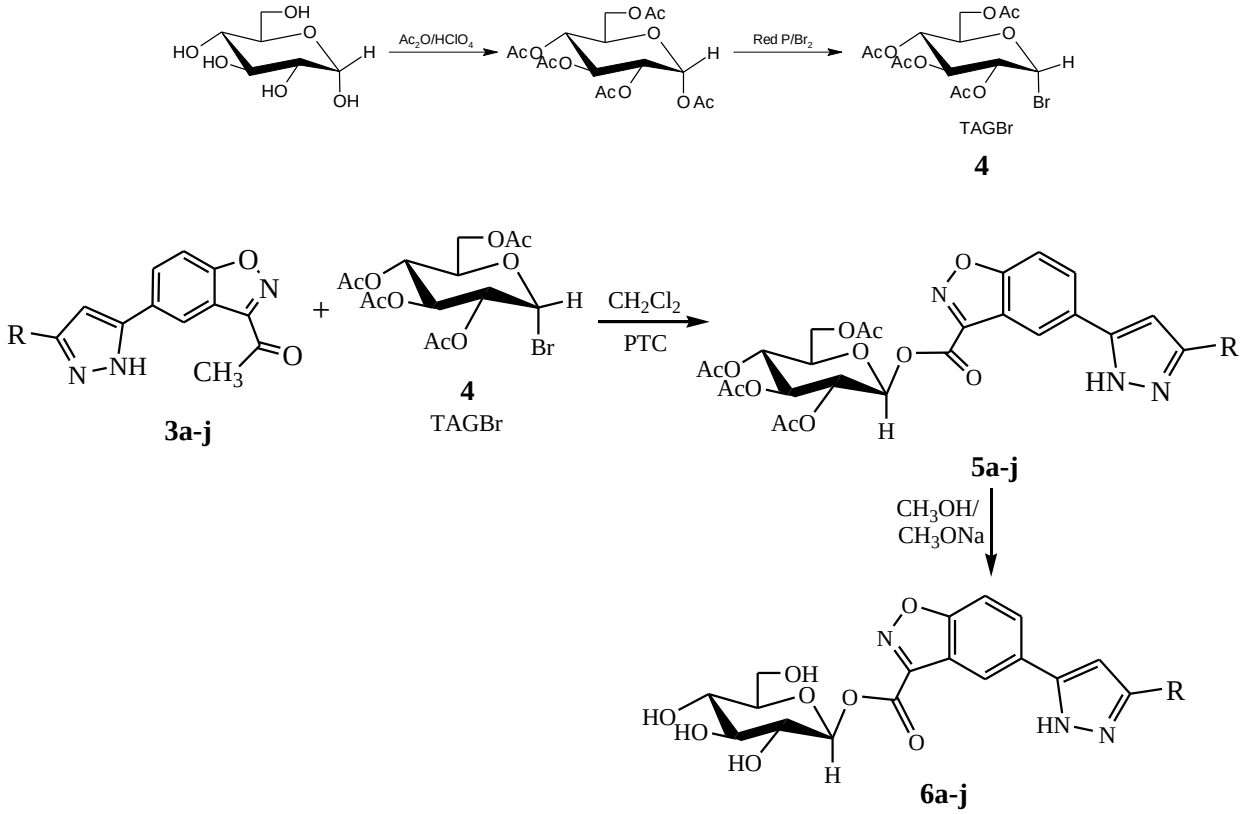

Scheme 2. Synthesis of *O*-Glucosides

3.2. Biological assay

The compounds **6a-j** were tested for their potential growth inhibitory activity against *Bacillus subtilis* and *Escherichia coli* at concentration of 100µg/mL in DMF. Norfloxacin was used as standard antibiotic for the comparison of the results. The sterilized Mullier-Hinton agar medium 50mL was inoculated with test organism and poured into petridishes. The four holes of 6mm were completely filled with different test solution. The plates were then incubated for 24hrs at 37^0C and zones of inhibitions were measured. Similar procedure was adopted for pure Norfloxacin and the corresponding zone diameter were compared. According to results, compounds **6a,b,c,e,f** showed activity against both organisms. Addition compound **6b and 6f** are higher active than compound **6a** against both organisms. The results are given in **Table 3**.

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Table 3. Data for in vitro antibacterial and antifungal activities of compounds **6a-j**.

	Diameter of Inhibition Zone (in mm) against			
	Bacterial Strains		**Fungal Strain**	
Products	***E. Coli***	***B. subtilis***	***A. niger***	***C. albicans***
6a	14	15	22	25
6b	16	15	27	28
6c	15	13	20	23
6d	12	10	18	17
6e	16	13	21	23
6f	16	16	25	27
6g	--	16	16	19
6h	14	--	22	18
6i	13	09	15	24
6j	12	14	20	21

-- = 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 *B. subtilis* 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.

4. Conclusion

A new series of carboxylic acids and their *O*-glucoside derivatives containing 1,2-benzisoxazoles and pyrazoles moieties have been prepared. The *O*-glucosides, (2S,3S,4S,5S)-tetrahydro-3,4,5-trihydroxy-6-(hydroxymethyl)-2H-pyran-2-yl-5-(3-aryl-1H-pyrazol-5-yl)benzo[d]isoxazole-3-carboxylate **6a-j** were synthesized and these products were evaluated for in vitro antibacterial activity against *Escherichia coli* and *Bacillus subtilis* strain as well as for antifungal activity against *Candida allbicans* and *Aspergilus niger* strain using cup-plate method. Various *O*-glucoside derivatives show excellent results against bacterial and fungal strains. The structures of the newly products have been assigned on the basis of FT-IR spectra, ^{1}H NMR, ^{13}C NMR, FAB-MS, optical activity and elemental analysis.

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Table 1. Physicochemical data of the synthesized compounds (**2a-j**)

Comp	R	Molecular Formula	Molecular Weight	R_f Value	Found (Calculated) %		
					C	H	N
2a	C_6H_5	$C_{17}H_{13}N_3O$	275	0.24	74.15 (74.17)	4.75 (4.76)	15.25 (15.25)
2b	*o*-OHC_6H_4	$C_{17}H_{13}N_3O_2$	291	0.33	70.11 (70.09)	4.52 (4.50)	14.40 (14.42)
2c	2,4-$(OH)_2C_6H_3$	$C_{17}H_{13}N_3O_3$	307	0.31	66.41 (66.44)	4.25 (4.26)	13.66 (13.67)
2d	*p*-OH-*m*-$OCH_3C_6H_3$	$C_{18}H_{15}N_3O_3$	321	0.26	67.26 (67.28)	4.70 (4.71)	14.93 (14.94)
2e	*o*-ClC_6H_4	$C_{17}H_{12}ClN_3O$	309	0.22	65.90 (65.92)	3.89 (3.90)	13.56 (13.57)
2f	*o*-$NO_2C_6H_4$	$C_{17}H_{12}N_4O_3$	320	0.32	63.74 (63.75)	3.78 (3.78)	17.48 (17.49)
2g	2-C_5H_4N	$C_{16}H_{12}N_4O$	276	0.29	69.54 (69.55)	4.37 (4.38)	20.19 (20.28)
2h	3-C_4H_3O	$C_{15}H_{11}N_3O_2$	265	0.30	67.92 (67.92)	4.35 (4.18)	15.83 (15.84)
2i	3-C_8H_5N	$C_{19}H_{14}N_4O$	314	0.22	72.59 (72.60)	4.48 (4.49)	17.81 (17.82)
2j	*p*-*N*$(CH_3)_2C_6H_4$	$C_{19}H_{18}N_4O_2$	318	0.37	71.67 (71.68)	5.69 (5.70)	17.58 (17.60)

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Table 2. Physicochemical data of the synthesized compounds (**6a-j**)

Comp	R	Molecular Formula	$[\alpha]_D^{25}$ (^{0})	R_f Value	Found (Calculated) %		
					C	H	N
6a	C_6H_5	$C_{23}H_{21}N_3O_8$	+44.7	0.24	58.99 (59.10)	4.50 (4.53)	8.95 (8.99)
6b	*o*-OHC_6H_4	$C_{23}H_{21}N_3O_9$	+46.1	0.21	57.11 (57.14)	4.29 (4.38)	8.71 (8.69)
6c	2,4-$(OH)_2C_6H_3$	$C_{23}H_{21}N_3O_{10}$	+47.0	0.20	55.24 (55.31)	4.22 (4.24)	8.36 (8.41)
6d	*p*-OH-*m*-$OCH_3C_6H_3$	$C_{24}H_{23}N_3O_{10}$	+49.2	0.28	56.11 (56.14)	4.54 (4.52)	8.21 (8.18)
6e	*o*-ClC_6H_4	$C_{23}H_{20}ClN_3O_8$	+47.3	0.31	55.93 (55.04)	4.29 (4.02)	8.40 (8.37)
6f	*o*-$NO_2C_6H_4$	$C_{23}H_{20}N_4O_{10}$	+48.1	0.22	53.85 (53.91)	3.93 (3.93)	10.8 (10.9)
6g	2-C_5H_4N	$C_{22}H_{20}N_4O_8$	+45.1	0.18	56.43 (56.41)	4.31 (4.30)	11.8 (11.9)
6h	3-C_4H_3O	$C_{21}H_{19}N_3O_9$	+41.9	0.26	56.11 (56.14)	4.17 (4.19)	9.14 (9.19)
6i	3-C_8H_5N	$C_{25}H_{22}N_4O_8$	+50.3	0.28	59.27 (59.29)	4.25 (4.38)	11.0 (11.2)
6j	*p*-*N*$(CH_3)_2C_6H_4$	$C_{25}H_{26}N_4O_8$	+50.8	0.24	58.81 (58.82)	5.10 (5.13)	10.8 (10.9)

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