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Design, synthesis and antiinflammatory activity of novel phthalimide derivatives, structurally related to thalidomide

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Abstract—As part of an ongoing effort to develop new thalidomide analogues as antiinflammatory lead-candidates, this paper describes the synthesis and antiinflammatory activity of novel *N*-phenyl-phthalimide functionalized derivatives (**4a**–**d**, **5a**,**b**, **6a**,**b**). The target compounds were assayed in an acute lung inflammatory model and all compounds were able to inhibit TNF-α production and subsequent neutrophil recruitment in the LPS-acute lung inflammatory model.

1. Introduction

Thalidomide (**1**), first synthesized as an antihistaminic drug in 1954, was introduced as a sedative/hypnotic drug in 1956 but withdrawn from the market because of its catastrophic teratogenicity.[1] In the early 1960s, a new use was found for thalidomide (**1**) as a sedative in patients suffering from lepromatous leprosy (*erythema nodosum leprosum*, ENL). A rapid and noticeable improvement of the painful neuritis experienced by these patients was observed and published by Sheskin in 1965.[2] This activity was a posteriori attributed to a selective blockade of tumor necrosis factor-α (TNF-α) production,[3] suggesting a new use for thalidomide (**1**) as antiinflammatory and immunomodulator,[3,4] under restricted conditions.

TNF-α is a cytokine considered to be a primary mediator of the inflammatory response.[5] At lung level, it has been demonstrated that the release of TNF-α favors the sequestration and migration of neutrophils which play a critical role in the pathogenesis of lung inflammation.[6–8] To modulate the over production of TNF-α, associated to the severity of many pathological processes, different strategies has been employed.[9–11] In this context, new synthetic thalidomide analogues, designed for keeping its beneficial action and avoiding its side effects, have been developed. We have described previously the identification of the prototypes **2** (LASSBio 468) and **3** (LASSBio 595) (Chart 1), designed as hybrid analogues of thalidomide (**1**) and aryl-sulfonamide phosphodiesterase inhibitors, which presented antiinflammatory properties acting on TNF-α production.[12]

In the present paper, we investigate the antiinflammatory effect of new phthalimide derivatives planned as a new generation of analogues of the lead-compounds **2** and **3** (Chart 1).

As depicted in Chart 1, compound **4d** represents the interphenylene analogue of **4a**–**c**, which presents a distinct substituent pattern in the phenyl-phthalimide moiety in comparison with the initial lead compounds **2** and **3**, through the introduction of a *ortho*-phenoxy-ester motif. In addition, derivative **5b** was designed as a hybrid of the compounds **4b** and **5a**, while compound **6b** is also a hybrid analogue planned from the isosteric leads **2** and **4b**.

As outlined in Scheme 1, the key step to prepare our target molecules (**4a**–**d**, **5a** and **b**, **6a** and **b**) involved the

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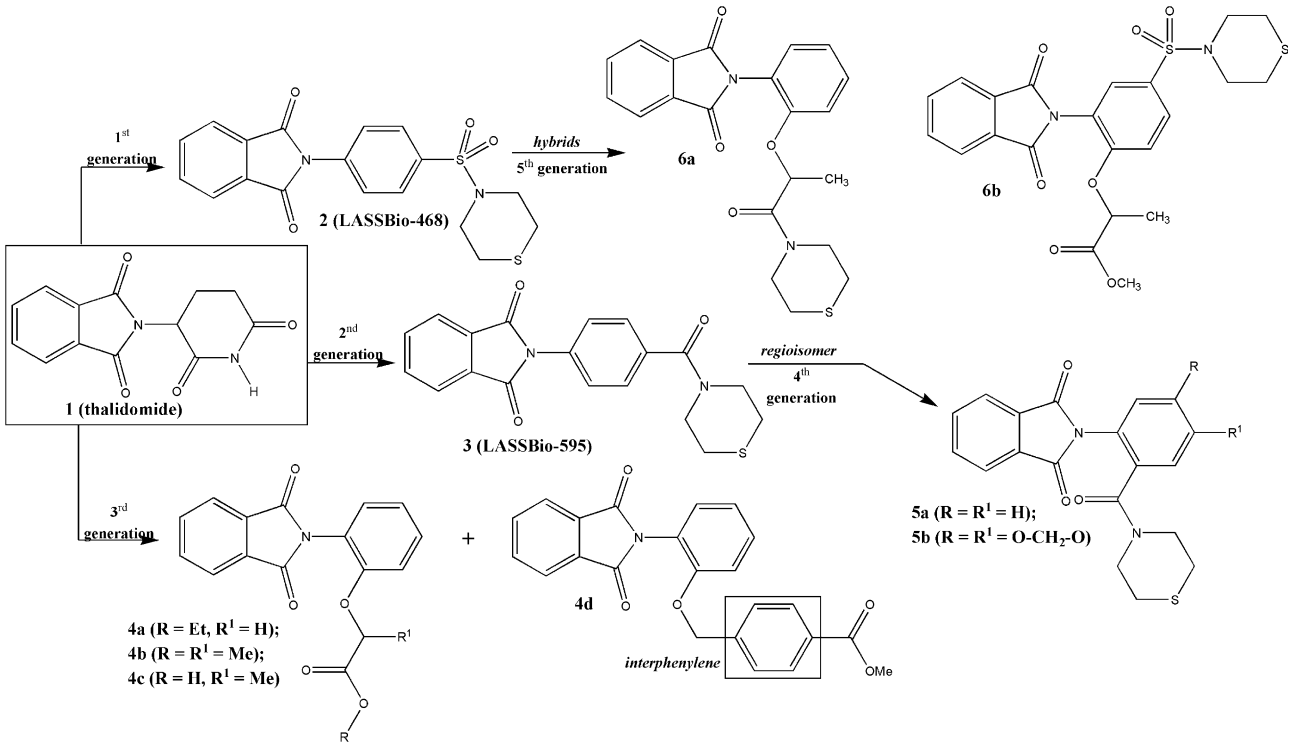
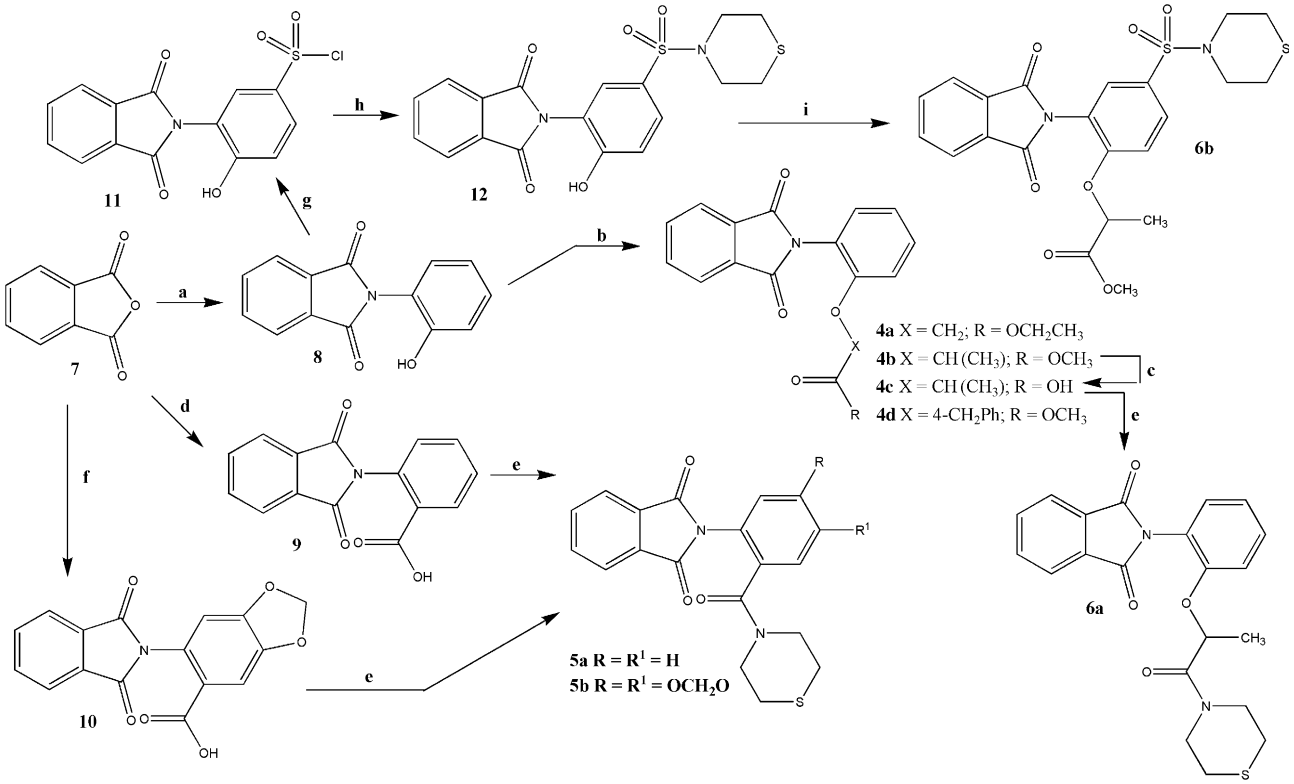


Chart 1.



Scheme 1. Conditions: (a) 2-NH_2PhOH, AcOH, reflux, 1 h, 91%; (b) EtO_2CCH_2Br or $MeO_2CCH(CH_3)Br$ or MeO_2CPhCH_2Br, K_2CO_3, DMF, rt, 72 h, 74–83%; (c) HCl:AcOH (1:1), rt, 1 h, 85%; (d) 2-NH_2PhCO_2H, AcOH, reflux, 1 h, 83%; (e) 1-$SOCl_2$, DMF (cat.), reflux, 1 h; 2-thiomorpholine, CH_2Cl_2, rt, 1 h, 80% (**5a**), 85% (**6a**) and 87% (**6b**); (f) 6-aminobenzo[*d*][1,3]dioxole-5-carboxylic acid, AcOH, reflux, 1 h, 80%; (g) $ClSO_3H$, 90 °C, 2 h, 89%; (h) thiomorpholine, CH_2Cl_2, rt, 2 h, 78%; (i) $MeO_2CCH(CH_3)Br$, K_2CO_3, DMF, rt, 72 h, 60%.

easy condensation of phthalic anhydride (**7**) with the corresponding functionalized amines at reflux.[13] By applying this approach, it was possible to obtain the *ortho* and *meta N*-phenyl-phthalimide substituted deriva-

tives (**8**–**10**) in high yields. With these intermediates in hands, we accomplished the *O*-alkylation of compound **8** with the adequately functionalized alkyl bromide, using K_2CO_3 in DMF at room temperature, which resulted in the formation of ester derivatives **4a**, **4b**, and **4d** in good yields.[14] Further, the ester **4b** was hydrolyzed by treatment with a mixture of HCl and AcOH (1:1)[15] furnishing compound **4c** in 85% yield.

The target amide derivatives **5a**,**b** and **6a** were prepared from functionalized phthalimides **9**, **10**, and **4c**, in excellent yields, using thionyl chloride to convert its carboxylic acid moiety in the corresponding acyl chloride intermediates, followed by treatment with thiomorpholine in dichloromethane at room temperature.[16] Finally, the synthesis of sulfonamide derivative **6b** involved regioselective eletrophilic aromatic substitution of phthalimide **8**, employing chlorosulfonic acid at 60 °C, obtaining the sulfonylchloride intermediate **11** in 89% yield, which was next condensed with thiomorpholine to give **12**.[11] *O*-alkylation of **12** with methyl 2-bromopropanoate in the presence of K_2CO_3 in DMF at room temperature furnished **6b** in 60% yield, as showed in Scheme 1.[17,18]

Groups of seven mice were treated ip with 10 mg/kg of thalidomide or its analogues (**4a** and **d**, **5a** and **b**, **6a** and **b**) 1 h before inhalation of aerosols of LPS.[12] Treatment with all drugs inhibited both TNF-α and neutrophil influx into mouse lungs as showed in Figure 1.

In the series of 2-phenoxy-phthalimide derivatives (**4a**–**d**), the racemate **4b** was the most active, inhibiting 60% of TNF-α production and neutrophil influx, similar to the effect of thalidomide. These results indicate that the substitution in the *ortho* position of the phenyl ring of *N*-phenyl-phthalimide moiety led to the same inhibitory profile as functionalization in the *para* position (e.g., **3**).[12] For this reason, in the next step the activity of the target phthalimide-amides **5a** and **5b** were evaluated with the purpose of investigating the contribution of the methylenedioxy subunit in the antiinflammatory activity. The data obtained showed that these compounds were equipotent to the prototype **3** (LASSBio 595, i.e., 48% inhibition of the neutrophils recruitment induced by LPS[12]) as inhibitors of the inflammatory response. Taken together, these data indicate that the antiinflammatory profile is closely dependent of the

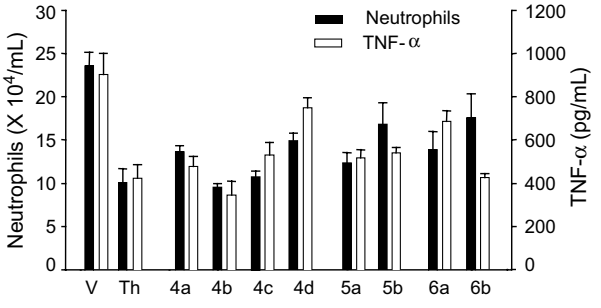


Figure 1. Inhibitory effect of thalidomide and its analogues on TNF-α production and neutrophil influx into the BALF of mice lungs. V = vehicle or control; Th = thalidomide.

substitution pattern in the phenyl ring, considering that no activity for *N*-phenyl-phthalimide was found (data not shown). Furthermore, these data indicate that substitutions in the positions *ortho* or *para*, in the phenyl ring, for sulfonamide (**2**), amide, (**3**,**5a**,**b**, ester **4a**,**b**,**d**), or either by carboxylic acid (**4c**) are essential for the antiinflammatory activity, through the modulation of the production of TNF-α. To investigate a possible optimization of the antiinflammatory activity of these compounds, a double functionalization in the *N*-attached phenyl ring produced the compound **6b** (LASSBio 867), designed as an hybrid derivative between **2** (LASSBio 468) and **4b** (LASSBio 542). Treatment with 10 mg/kg of compound **6b** 1 h before inhalation of LPS did not improve the antiinflammatory activity, even considering the presence of the sulfonamide group in C-3 and the phenoxy-ester group in C-2 positions.

Our data show, for all the compounds tested, that the inhibition of TNF-α production was accompanied by a similar reduction in neutrophil recruitment triggered by aerosols of LPS into alveolar spaces of mouse lungs. However, the functionalization in more than one position in the phenyl ring of phthalimide system, such as for **5b** and **6b**, led to a decrease in the inhibitory effect on TNF-α levels and neutrophil recruitment.

In conclusion, in an effort to discover new thalidomide analogues with antiinflammatory activity we were able to identified novel *N*-phenyl-phthalimide derivatives that blocked the inflammatory reaction in mouse lungs triggered by aerosols of LPS, similar to the prototype thalidomide.

Acknowledgments

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17. The analytical results for C, H, N of the compounds **4a**–**d**, **5a**,**b**, and **6a**,**b** were within ±0.4% of calculated values.
18. Physical and spectroscopic data: Compound **4a**: mp: 137–138 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.15 (t, J = 7.14 Hz, $ROCH_2CO_2CH_2CH_3$); 4.15 (q, J = 7.14 Hz, $ROCH_2CO_2CH_2CH_3$); 4.62 (s, $ROCH_2CO_2CH_2CH_3$); 6.97 (dd, J = 8.33 Hz and 1.10 Hz, H3′); 7.15 (dt, J = 7.60 Hz and 7.60 Hz, H5′); 7.32 (dd, J = 7.78 Hz and 1.74 Hz, H6′); 7.42 (dt, J = 8.30 Hz and 7.51 Hz, H4′); 7.78 (m, H5 and H6); 7.95 (m, H4 and H7); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 14.08 ($ROCH_2CO_2CH_2CH_3$); 61.42 ($ROCH_2CO_2CH_2CH_3$); 66.50 ($ROCH_2CO_2CH_2CH_3$); 113.84 (C3′); 121.37 (C1′); 122.34 (C5′); 123.81 (C7 and C4); 130.35 (C4′); 130.65 (C6′); 132.41 (C7a and C3a); 134.26 (C6 and C5); 154.08 (C2′); 167.33 (C1 and C3); 168.38 ($ROCH_2CO_2CH_2CH_3$); Compound **4b**: mp: 101–102 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.42 (d, J = 6.80 Hz, $ROCH(CH_3)CO_2CH_3$); 3.59 (s, $ROCH(CH_3)CO_2CH_3$); 4.69 (q, J = 6.80 Hz, $ROCH(CH_3)CO_2CH_3$); 6.86 (d, J = 8.17 Hz, H3′); 7.05 (dt, J = 7.44–7.70 Hz, H5′); 7.23 (dd, J = 7.71–1.50 Hz, H6′); 7.32 (dt, J = 8.20–7.48 Hz, H4′); 7.72 (m, H5–H6); 7.83 (m; H4–H7); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 18.47 ($ROCH(CH_3)CO_2CH_3$); 52.33 ($ROCH(CH_3)CO_2CH_3$); 73.99 ($ROCH(CH_3)CO_2CH_3$); 114.23(C3′); 121.42 (C1′); 122.08 (C5′); 123.59–123.70 (C7–C4); 130.30 (C6′); 130.54 (C4′); 132.15–132.33 (C3a–C7a); 134.19 (C6–C5); 153.79 (C2′); 167.14–167.23 (C1–C3); 171.99 ($ROCH(CH_3)CO_2CH_3$); Compound **4c**: mp: 125–126 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.56 (d, J = 6.76 Hz, $ROCH(CH_3)CO_2H$); 4.94 (q, J = 6.77 Hz, $ROCH(CH_3)CO_2CH_3$); 5.75 (s, CO_2 *H*); 7.01 (d, J = 8.20 Hz, H3′); 7.16 (dt, J = 7.53 Hz and 7.58 Hz, H5′); 7.35 (d, J = 8.65 Hz, H6′); 7.42 (dt, J = 7.58 Hz and 8.70 Hz, H4′); 7.83 (m, H5 and H6); 7.97 (m, H4 and H7); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 18.48 ($ROCH(CH_3)CO_2H$); 73.25 ($ROCH(CH_3)CO_2H$); 113.03 (C3′); 120.52 (C1′); 122.60 (C5′); 124.22 (C7 and C4); 125.92 (C6′); 130.83 (C4′); 131.98 and 132.03 (C7a and C3a); 134.83 (C6 and C5); 151.92 (C2′); 167.11 and 168.24 (C1 and C3); 173.15 ($ROCH(CH_3)CO_2H$); Compound **4d**: mp: 165–167 °C;^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.83 (s; $ArCO_2CH_3$); 5.19 (s, $ROCH_2Ar$); 7.02 (dd, J = 8.30 Hz and 1.18 Hz, H3′); 7.12 (dt, J = 7.62 Hz and 7.68 Hz, H5′); 7.32 (dd, J = 7.87 Hz and 1.78 Hz, H6′); 7.38 (d, J = 8.13 Hz, H2′′ and H6′′); 7.39 (m, H4′); 7.79 (m, H5 and H6); 7.94 (d, J = 8.00 Hz, H3′′ and H5′′); 7.95 (m, H4 and H7); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 51.90 (RCO_2CH_3); 69.57 ($ROCH_2R$); 113.32 (C3′); 120.63 (C1′); 121.30 (C5′); 126.38 (C2′′ and C6′′); 123.46 (C7 and C4); 129.35 (C4′′); 129.59 (C3′′ and C5′′); 129.99 (C4′); 130.41 (C6′); 131.98 (C7a and C3a); 134.06 (C6 and C5); 141.51 (C1′′); 153.93 (C2′); 166.57 (RCO_2CH_3); 167.17 (C1 and C3); Compound **5a**: mp: 139–140 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.58 (m, CH_2S); 3.73 (m, CH_2N); 8.13 (d, J = 8.20 Hz, H3′); 7.29 (dt, J = 7.78 Hz and 8.70 Hz, H5′); 7.45 (d, J = 8.70 Hz, H6′); 7.22 (dt, J = 7.78 Hz and 8.20 Hz, H4′); 7.85 (m, H5 and H6); 7.91 (m, H4 and H7); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 28.02 (CH_2S); 28.30 (CH_2S); 44.99 (CH_2N); 49.68 (CH_2N); 129.83 (C3′); 141.52 (C1′); 131.60 (C5′); 123.92 (C7 and C4); 121.89 (C6′); 126.88 (C4′); 131.98 and 137.03 (C7a and C3a); 134.60 (C6 and C5); 122.83 (C2′); 165,61 ($ArCONR_2$); 166.85 and 167.94 (C1 and C3); Compound **5b**: mp: 159–160 °C;^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.53 (m, CH_2S); 3.70 (m; CH_2N); 6.01 (s, OCH_2O); 6.63 (s, H3′); 7.08 (s, H6′); 7.86 (m, H5 and H6); 7.89 (m, H4 and H7); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 28.06 (CH_2S); 29.10 (CH_2S); 44.50 (CH_2N); 49.02 (CH_2N); 100.61 (OCH_2O); 107.88 (C3′); 135.48 (C1′); 149.74 (C5′); 123.71 (C7 and C4); 104.89 (C6′); 148.88 (C4′); 131.98 and 137.83 (C7a and C3a); 137.90 (C6 and C5); 119.11 (C2′); 164,91 ($ArCONR_2$); 166.80 and 166.95 (C1 and C3); Compound **6a**: brownish oil; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.51 (d; J = 6.76 Hz, $ROCH(CH_3)CONR$); 2.60 (m; CH_2S); 3.70 (m; CH_2N); 4.90 (q, J = 6.77 Hz, $ROCH(CH_3)CONR$); 7.02 (d, J = 8.20 Hz, H3′); 7.18 (dt, J = 7.53 Hz and 7.58 Hz, H5′); 7.36 (d, J = 8.65 Hz, H6′); 7.44 (dt, J = 7.58 Hz and 8.70 Hz, H4′); 7.85 (m; H5 and H6); 7.94 (m; H4 and H7); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 18.44 ($ROCH(CH_3)CONR$); 27.44 (CH_2S); 28.31 (CH_2S); 44.87 (CH_2N); 48.26 (CH_2N); 72.85 ($ROCH(CH_3)CONR$); 113.01 (C3′); 120.55 (C1′); 122.70 (C5′); 124.45 (C7 and C4); 126.02 (C6′); 130.83 (C4′); 131.98 and 132.05 (C7a and C3a); 134.61 (C6 and C5); 151.95 (C2′); 166,28 ($ArOCH(CH_3)CONR$); 167.15 and 168.21 (C1 and C3); Compound **6b**: brownish oil; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.53 (d, J = 6.70 Hz, $ROCH(CH_3)CONR$); 2.77 (m, CH_2S); 2.81 (m, CH_2 S); 3.68 (m, CH_2N); 3.90 (m, CH_2N); 4.88 (q, J = 6.70 Hz, $ROCH(CH_3)CONR$); 7.09 (d, J = 8.10 Hz, H3′); 8.08 (s, H6′); 8.12 (d, J = 8.10 Hz, H4′); 7.93 (m, H5 and H6); 7.97 (m, H4 and H7); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 19.40 ($ROCH(CH_3)CONR$); 27.40 (CH_2S); 27.45 (CH_2S); 27.85 (CH_2S); 28.05 (CH_2S); 44.85 (CH_2N); 48.26 (CH_2N); 50.81(CH_2N); 51.05 (CH_2N); 73.01 ($ROCH(CH_3)CONR$); 115.43 (C3′); 131.12 (C1′); 139.60 (C5′); 124.72 (C7 and C4); 115.95 (C6′); 126.77 (C4′); 131.98 and 134.53 (C7a and C3a); 134.60 (C6 and C5); 154.10 (C2′); 168.01($ArOCH(CH_3)CONR$); 167.11 and 167.23 (C1 and C3).