Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 171–175

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Solid-Phase Synthesis of New Saphenamycin Analogues with Antimicrobial Activity

Jane B. Laursen, Peter C. de Visser,† Henrik K. Nielsen, Knud J. Jensen‡ and John Nielsen*

Department of Chemistry, Kemitorvet, Building 207, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark

Received 12 July 2001; revised 5 October 2001; accepted 17 October 2001

Abstract—An array of 12 new saphenamycin analogues modified at the benzoate moiety was synthesized on solid support. Synthesis commenced with a chemoselective anchoring of saphenic acid through the carboxyl group to a 2-chlorotrityl functionalized polystyrene resin. The secondary alcohol was acylated in parallel with a series of differently substituted benzoic acid derivatives. Treatment with TFA–CH_2Cl_2 (5:995) released the expected saphenamycin analogues into solution. These new analogues were purified, characterized and screened for antimicrobial activity against *Bacillus subtilis* and *Proteus mirabilis*. Eight analogues exhibited MIC values against *B. subtilis* ranging from 0.07 to 3.93 μg/mL, comparable to the activities of previously reported saphenamycin analogues.

Introduction

The search for new antiviral and antibacterial compounds has accelerated in recent years due to the evident prevalence of antibiotic resistance. Thus, in the development of antibiotics as well as anticancer drugs, attention has been drawn towards a series of new targets, one of these being regulation of cell growth at the DNA/RNA level. A large group of antibiotics affect protein synthesis or nucleic acid metabolism, for example via interference with DNA replication, DNA transcription, aminoacyl–*t*RNA formation or RNA translation.[1] Intercalation, that is formation of a non-covalent complex of the drug with the duplex DNA, will result in inhibition of DNA replication and/or transcription, presumably due to deformation of the double helix.[2]

Many intercalating drugs contain planar aromatic ring systems with cationic moieties. Polyaromatic rings are able to stack between the base pairs of the duplex, whereas the positively charged groups can stabilize the interaction by ion-pair formation with the negatively charged phosphate backbone.[3] Several naturally occurring antibiotics bind to DNA, by either minor or major groove binding (e.g., distamycin A),[4] by intercalation (e.g., ethidium bromide, proflavine and quinacrine)[2] or by both (e.g., daunomycin).[5] Intercalating drugs have been known since World War II, when acridine analogues were used against malaria infections. Today, intercalating substances including some medicinal drugs are known to have a wide range of indications, including antimalarial (quinacrine), antimicrobial (saphenamycin), antitumor (daunomycin), antitrypanosomal (ethidium bromide) and antineoplastic (anthracyclines).[2,4,5]

In the 1980's, a group of potential antibiotics containing the planar tricyclic heteroaromatic phenazine was isolated from the marine microorganism *Streptomyces antibioticus* strain Tü 2706.[6] The derivatives included the diphenazine antibiotics Esmeraldin A and B as well as simpler monomeric structures containing 6-(1-hydroxyethyl)-1-phenazine carboxylic acid, *saphenic acid* (**1**, Fig. 1), mainly as their saphenyl esters. Especially the 6-methylsalicylic acid saphenyl ester, *saphenamycin* (**2**, Fig. 1) has shown extensive antimicrobial activity towards a broad range of bacteria.[7–9] Also carbohydrate containing saphenic acid derivatives like the rare L-quinovosyl esters were isolated from marine micro-

*Corresponding author. Tel.: +45-4525-2131; fax: +45-4593-3968; e-mail: jni@kemi.dtu.dk

†Present address: Leiden Institute of Chemistry, Gorlaeus Laboratories, Leiden University, Einsteinweg 55, PO Box 9500, NL-2300 RA Leiden, The Netherlands.

‡Present address: Chemistry Department, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark.

0960-894X/02/$ - see front matter
PII: S0960-894X(01)00692-8

organisms and proven to inhibit growth of various types of bacteria and tumors.[10]

We have recently reported an efficient method for synthesis of racemic saphenic acid.[11] We envisioned that modifications on the benzoate moiety in saphenamycin might modulate or even enhance the biological activity and specificity compared to the naturally occurring compounds. Previous reports on antitumor and antibiotic activity of some saphenic acid esters have shown enhanced activity of esters of aromatic acids compared to those of aliphatic acids.[8] This indicates that the substituted benzoate moiety might be crucial for the biological activity. Furthermore, it was reported[8] that removal of the 6″-methyl group of saphenamycin had only a minor effect on bioactivity compared to the natural compound, whereas removal of the 2″-hydroxyl group in these cases lowered bioactivity. These results imply that the benzoate moiety and the 2″-hydroxyl group are important pharmacophoric elements.

Herein, we report a facile and expeditious solid-phase synthesis of saphenamycin derivatives. Following spectroscopic identification, these analogues were subjected to plate-diffusion tests against *Bacillus subtilis* and *Proteus mirabilis* and serial dilution tests on solid media to determine minimal inhibiting concentration (MIC) against *B. subtilis*.[1]

Synthesis

Our synthetic approach to the parallel synthesis of saphenamycin analogues included (i) chemoselective anchoring of saphenic acid through its carboxyl group, (ii) acylation of the secondary hydroxyl with benzoic acid derivatives, and (iii) release of products by treatment with mild acid (TFA–CH_2Cl_2, 5:995)[12] compatible with the acid-labile C1′–O bond (Scheme 1).

In the first step, saphenic acid was anchored to a 2-chlorotrityl (Clt) polystyrene (PS) resin in the presence of DIEA with 80% attach-release efficiency. Unreacted sites were capped by treatment with MeOH in the presence of DIEA. The 2-chlorotrityl resin served as a chemoselective carboxy protecting group in the following acylation of the secondary alcohol. Next, 12 different benzoic acid derivatives were chosen as analogues of 6-methylsalicylic acid, representing a diverse set of electronic and sterical properties (**3**–**14**, Table 1). Fluorine was included as substituent due to its electronic similarity with the hydroxyl group.[14] Chlorine was included for comparison and for its relative prevalence in antiinfectives and antiseptics.[15]

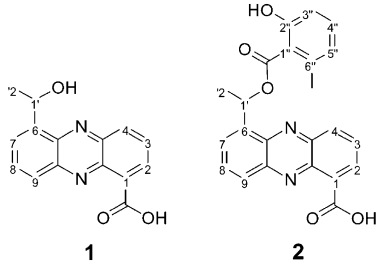
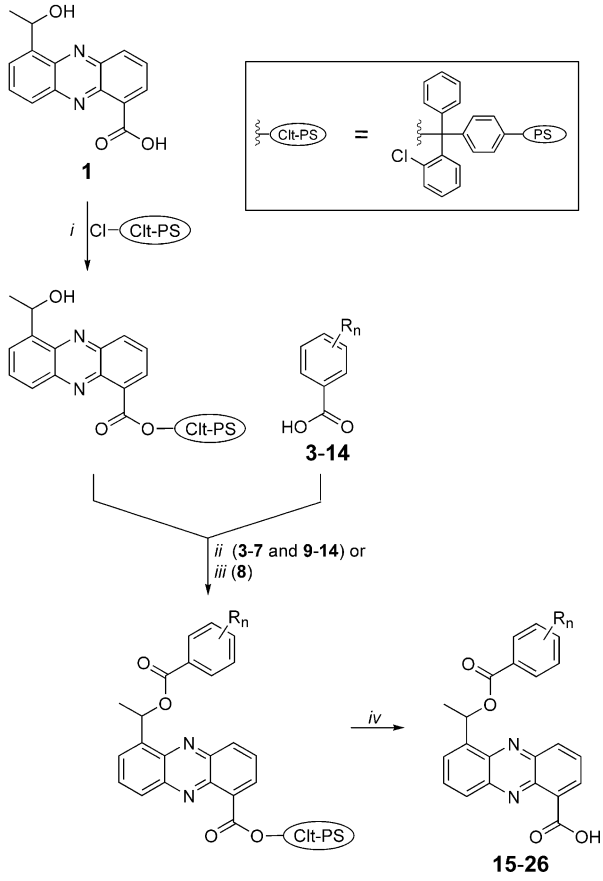


Figure 1. Structures of saphenic acid (**1**) and saphenamycin (**2**) and general numbering of atoms.

Benzoic acid derivatives **6**–**14** were commercially available, whereas derivatives **3**–**5** were synthesized by three



Scheme 1. Reagents and conditions (see also ref 13): (i) DIEA (4 equiv), CH_2Cl_2, rt, 16 h; (ii) $(COCl)_2$ (1 equiv), DMF (cat), CH_2Cl_2/DIEA, rt, 1 h, then DMAP (2.5 equiv), CH_2Cl_2, rt 16 h; (iii) NT (2 equiv), DCC (2 equiv), DMAP (1 equiv), rt, 16 h; (iv) TFA–CH_2Cl_2 (5:995), rt, 40 min. Overall yields ranged from 40 to 100%.

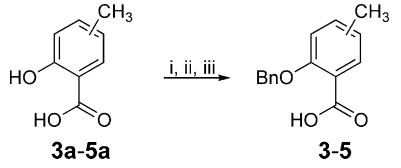
Table 1. Substituents on benzoic acid derivatives **3a**–**5a**, **3**–**14** and esters **2**, **15**–**26**

Substituent(s)	Acids	Esters
2″-Hydroxy-6″-methyl		**2**
2″-Hydroxy-3″-methyl	**3a**	
2″-Hydroxy-4″-methyl	**4a**	
2″-Hydroxy-5″-methyl	**5a**	
2″-Benzyloxy-3″-methyl	**3**	**15**
2″-Benzyloxy-4″-methyl	**4**	**16**
2″-Benzyloxy-5″-methyl	**5**	**17**
2″-Fluoro	**6**	**18**
3″-Fluoro	**7**	**19**
4″-Fluoro	**8**	**20**
2″,6″-Difluoro	**9**	**21**
2″-Chloro	**10**	**22**
3″-Chloro	**11**	**23**
2″,6″-Dichloro	**12**	**24**
2″,4″,6″-Trichloro	**13**	**25**
2″-Nitro	**14**	**26**

trivial chemical steps starting from methylsalicylic acid derivatives **3a**–**5a** (Scheme 2).

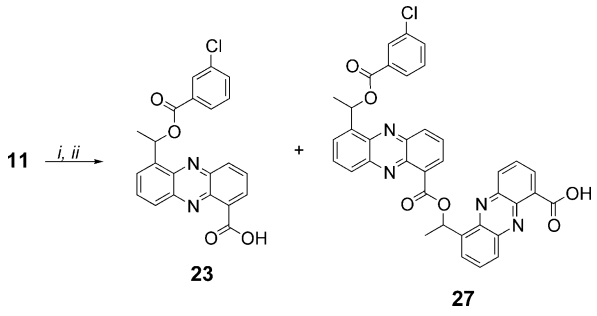
Initially, the acids were converted into the corresponding methyl esters by treatment with H_2SO_4 in refluxing MeOH.[16] Subsequently, the phenols were benzylated in good yields without prior purification using BnCl and K_2CO_3. Finally, saponification of the methyl esters gave the benzoic acid derivatives **3**–**5** in good yields.

Next, several methods for esterification with the benzoic acid derivatives were studied. Generally, acyl chlorides proved very efficient. They were prepared in situ by treatment of the benzoic acid derivative with oxalyl chloride and a catalytic amount of DMF in DIEA/CH_2Cl_2 to form a reactive Vilsmeier salt.[17,18] The acid chloride was added to resin-bound saphenic acid along with DMAP.[19] In one case, an alternative procedure, comprising *N,N′*-dicyclohexylcarbodiimide (DCC) and 3-nitrotriazole (NT), was used to prepare ester **20**.[20] Esterifications proceeded in moderate to excellent yields; repetition of acyl chloride esterifications increased the yield by only 2–3%. Cleavage from the resin proceeded smoothly by treatment with TFA–CH_2Cl_2 (5:995) releasing the saphenamycin analogues **15**–**26**, which were subsequently purified by silica column chromatography and isolated as yellow, green or brown amorphous solids.



3a-5a **3-5**

Scheme 2. Reagents and conditions: (i) MeOH, concd H_2SO_4 (cat), 70 °C; (ii) BnCl, K_2CO_3 (anhydr), DMF, 63–85% (two steps); (iii) NaOH, THF/H_2O, 73–89%.



23 **27**

Scheme 3. Reagents and conditions: (i) $(COCl)_2$ (1 equiv), DMF (cat), CH_2Cl_2, rt, 1 h, then DMAP (2.5 equiv), **1** (1 equiv), CH_2Cl_2, rt 16 h; isolated yields: 35% **23**, 20% **27**.

After purification, the purity was determined by reversed phase HPLC analysis. The identity of the 12 saphenamycin analogues and byproduct **27** was confirmed by NMR[22] and high resolution mass spectrometry (HR-MS, Table 2) and the compounds were finally tested for biological activity.

Biological activity

Compounds **15**–**27** were screened for their ability to inhibit growth of Gram-positive *B. subtilis* and Gram-negative *P. mirabilis*. All analogues were subjected to an

Compound **23**, which proved most active (vide infra), was resynthesized in larger scale (0.75 mmol) in solution from saphenic acid using conditions similar to those used on solid phase but without protection of the saphenic acid carboxylate. The lack of a carboxyl protecting group led to formation of diphenazine byproduct **27**[21] and the isolated yield of **23**[21] was significantly reduced (Scheme 3). This supported our original strategy of protection of the carboxyl group by anchoring to a solid support.

Table 2. Yields and purities of esters **15**–**26** and byproduct **27** with HR-MS data

Compd	Yield (%)[a]	Purity (%)[b]	Formula	HR-MS data	
				Calcd	Found
15	Quant	94	$C_{30}H_{25}N_2O_5$	493.1763	493.1705
16	90	91	$C_{30}H_{25}N_2O_5$	493.1763	493.1712
17	90	90	$C_{30}H_{25}N_2O_5$	493.1763	493.1716
18	Quant	96	$C_{22}H_{16}FN_2O_4$	391.1094	391.1104
19	Quant	95	$C_{22}H_{16}FN_2O_4$	391.1094	391.1114
20	66	82	$C_{22}H_{16}FN_2O_4$	391.1094	391.1101
21	40	n.d.	$C_{22}H_{15}F_2N_2O_4$	409.1000	409.0972
22	Quant	85	$C_{22}H_{16}{}^{35}ClN_2O_4$	407.0799	407.0824
23	Quant	99	$C_{22}H_{16}{}^{35}ClN_2O_4$	407.0799	407.0825
			$C_{22}H_{16}{}^{37}ClN_2O_4$	409.0769	409.0755
24	Quant	84	$C_{22}H_{15}{}^{35}Cl_2N_2O_4$	441.0409	441.0442
			$C_{22}H_{15}{}^{37}Cl_2N_2O_4$	445.0350	445.0374
25	47	89	$C_{22}H_{14}{}^{35}Cl_3N_2O_4$	475.0019	474.9983
			$C_{22}H_{14}{}^{35}Cl_2{}^{37}ClN_2O_4$	476.9990	476.9941
			$C_{22}H_{14}{}^{35}Cl{}^{37}Cl_2N_2O_4$	478.9960	478.9926
26	Quant	87	$C_{22}H_{16}N_3O_6$	418.1039	418.0962
27	20	98	$C_{37}H_{25}{}^{35}ClN_4O_6$	657.1541	657.1608
			$C_{37}H_{25}{}^{37}ClN_4O_6$	659.1511	659.1532

[a]Determined as the phenazine conversion from HPLC chromatograms (256 nm) of cleaved reaction mixtures, relative to peak of saphenic acid (**1**).
[b]Determined from HPLC chromatograms (256 nm) of purified compounds.
n.d. = not determined.

initial plate-diffusion test to reveal antibiotic activity. Compounds **15**, **20** and **22–27** showed inhibition of the Gram-positive *B. subtilis* (Table 3) while none of the tested phenazines had any inhibitory effect on *P. mirabilis*. Most of the active analogues contained chloride or fluoride, whereas the substitution pattern of **15** had some resemblance with the natural antibiotic **2**.

The compounds showing antibacterial activity in the initial test were subjected to further analysis. MIC values against *B. subtilis* were determined on solid medium containing serial dilutions of the test compound. A suspension of bacteria was standardized at 2×10^3 colony forming units per mL (CFU/mL) and compounds **15**, **20** and **22–27** were dissolved in DMSO (0.2 mg/mL) and further diluted with 0.9% aq NaCl.

MIC values ranged from 0.07 to 3.96 μg/mL (Table 3), the most potent being 3″-chloro compound **23**. Previous reports had included MIC values of the naturally occurring saphenamycin and analogues,[6–8] but to the best of our knowledge, investigations of the antimicrobial activity of meta-substituted analogues had not yet been reported. Saphenamycin analogue **23** possessed activity approximately one order of magnitude from the reported MIC value of saphenamycin (0.001 μg/mL);[8] all MIC values determined are thus at least comparable to other aromatic ester analogues of saphenic acid[7] and much more potent than aliphatic esters of the phenazine.[8]

In conclusion, we have synthesized an array of 12 new saphenamycin analogues modified on the benzoate moiety by facile parallel solid-phase synthesis. One of the new analogues exhibited antibacterial activity within 70-fold of the naturally occurring saphenamycin, comparable to or better than those of other saphenamycin analogues reported in literature. The present results are promising and further work on antimicrobial analysis as well as syntheses and separation of enantiomers of various saphenamycin analogues are in progress.

Table 3. Results of plate-diffusion test and MIC values of the saphenamycin analogues against *Bacillus subtilis*

Compd	Plate-diffusion test[a]	MIC[b] (μg/mL)
15	+	3.73
16	No growth inhibition	n.d.
17	No growth inhibition	n.d.
18	No growth inhibition	n.d.
19	No growth inhibition	n.d.
20	+	3.93
21	No growth inhibition	n.d.
22	+	3.35
23	+ +	**0.07**[c]
24	+	2.71
25	+ +	2.10
26	+	2.49
27	+ +	0.72
DMSO control	No growth inhibition	n.d.

[a]Test compound added in 10 μL (max concentration 200 μg/mL); + indicates clearance zones; + + indicates strong, well defined clearance zones.
[b]Values are means of three experiments (max concentration 10 μg/mL).
[c]MIC value is written in bold to accentuate that compound **23** is the most active compound.
n.d. = not determined.

Acknowledgements

We are thankful to Professor Michael Givskov for assisting in the microbial assays. Financial support for the project was provided in part by the Technical University of Denmark (J.B.L.), the Torkil Holm Foundation (J.N.), the Villum Kann Rasmussen Foundation (J.N.) and the Alfred Benzon Foundation (K.J.J.).

References and Notes

1. Zähner, H.; Maas, W. K. In *Biology of Antibiotics*; Springer: New York, 1972; p 78.
2. Wilson, W. D.; Jones, R. L. In *Intercalation Chemistry*; Whittingham, M. S.; Jacobsen, A. J., Eds.; Academic: New York, 1982; p 445.
3. Yang, X.-L.; Wang, A. H.-J. *Pharm. Ther.* **1999**, *83*, 181.
4. Gabelica, V.; Pauw, E. D.; Rosu, F. *J. Mass Spectrom.* **1999**, *34*, 1328.
5. Kapur, A.; Beck, J. L.; Sheil, M. M. *Rapid Commun. Mass Spectrom.* **1999**, *13*, 2489.
6. Geiger, A.; Keller-Schierlein, W.; Brandl, M.; Zähner, H. *J. Antibiotics* **1988**, *41*, 1542.
7. Michel, K. H.; Hoehn, M. M. US Patent 4,316,959. *Chem. Abstr.* **1982**, *96*, 197888.
8. Bahnmüller, U.; Keller-Schierlein, W.; Brandl, M.; Zähner, H.; Diddens, H. *J. Antibiot.* **1988**, *41*, 1552.
9. Kitahara, M.; Nakamura, H.; Matsuda, Y.; Hamada, M.; Naganawa, H.; Maeda, K.; Umezawa, H.; Iitaka, Y. *J. Antibiot.* **1982**, *35*, 1412.
10. Pathirana, C.; Jensen, P. R.; Dwight, R.; Fenical, W. *J. Org. Chem.* **1992**, *57*, 740.
11. Petersen, L.; Jensen, K. J.; Nielsen, J. *Synthesis* **1999**, *10*, 1763.
12. Lloyd-Williams, P.; Albericio, F.; Giralt, E. *Tetrahedron* **1993**, *49*, 11065.
13. 2-Chlorotrityl (Clt) resin was purchased from Nova-Biochem (2% DVB on PS, loading capacity 0.67 and 1.24 mmol/g). MeOH, CH_2Cl_2, pentane, hexane and EtOAc were distilled prior to use. EtOAc, CH_2Cl_2 and DMF were dried over 4 Å molar sieves. Other solvents and reagents were used as delivered. Solid-phase loadings, conversion percentages and purities were calculated relative to the peak of **1** from HPLC analysis at 256 nm of cleavage mixtures from small amounts of resin (10 mg).

General procedure for coupling of 1 to resin. After drying over 4 Å molar sieves for 24 h the Clt resin (500 mg 0.67 or 1.24 mmol/g) was swelled in CH_2Cl_2 for 1 h in a silylated glass tube (20% TMS–Cl/$CHCl_3$ or SigmaCote). A solution of **1** (1.2 equiv) and DIEA (4 equiv) in CH_2Cl_2 was added to the resin and the reaction was stirred for 16 h. The resin was washed ($3\times CH_2Cl_2$–MeOH-DIEA 17:2:1, to cap unreacted Clt sites, $3\times CH_2Cl_2$, 2×DMF and $2\times CH_2Cl_2$). Loadings: 35–80%.

General procedure for formation of acid chlorides and coupling to on-resin 1. Benzoic acid derivative **3–7** or **9–14** (0.5 mmol, 1 equiv) was dissolved in CH_2Cl_2 (1.5 mL) and DIEA (0.25 mL). While stirring, $(COCl)_2$ (1 equiv) and DMF (catalytic amount) were added. The reaction was followed by dissolving a sample in MeOH and subsequent identification of

the methyl ester on TLC. Complete conversion was observed within 1 h. 1 mL of the solution was added to a silylated glass tube (see above) or plastic syringe containing on-resin **1** (0.1 equiv) and DMAP (0.66 equiv) in CH_2Cl_2 (1 mL). The mixture was shaken for 16 h and washed (2×DMF, 2×CH_2Cl_2). Conversions: 40–100%.

Procedure for formation of acid chloride of 8 and coupling to on-resin 1. Benzoic acid derivative **8** (115 μmol, 5 equiv) and 3-nitrotriazole (2 equiv) were dissolved in CH_2Cl_2 (DMF added to assist dissolvation). *N,N′*-Dicyclohexylcarbodiimide (2 equiv) and DMAP (1 equiv) were added and the mixture was poured into a silylated glass tube (see above) containing on-resin **1** (1 equiv). The reaction mixture was shaken for 16 h and washed (2×MeOH, 2×DMF and 3×CH_2Cl_2). The esterification was repeated. Conversion: 1st: 53%; 2nd: 66%.

General procedure for cleavage of derivatives 15–26 from resin. The resin was reacted with TFA–CH_2Cl_2 (5:995) for 40 min and washed (2×CH_2Cl_2).

14. Patani, G. A.; LaVoie, E. J. *Chem. Rev*. **1996**, *96*, 3147.
15. Foye, W. O.; Lemke, T. L.; Williams, D. A. In *Principles of Medicinal Chemistry*, 4th ed.; Balado, D. Ed.; Williams & Wilkins: Boston, 1995; pp. 759–821.
16. Marquerettaz, X.; O'Neill, R.; Fitzmaurice, D. *J. Am. Chem. Soc*. **1994**, *116*, 2629.
17. Wissner, A.; Grudzinskas, C. V. *J. Org. Chem*. **1978**, *43*, 3972.
18. Petersen, L.; Jensen, K. J. *J. Org. Chem*. **2001**, *66*, 6268.
19. Laursen, J. B.; Petersen, L.; Jensen, K. *J. Org. Lett*. **2001**, *3*, 687.
20. Nielsen, J.; Lyngsø, L. O. *Tetrahedron Lett*. **1996**, *37*, 8439.
21. In the up-scaled reaction, **23** and **27** were isolated as yellow crystalline compounds with mp 176–178 and 168–171 °C, respectively.
22. Selected ^{1}H NMR data ($CDCl_3$): **15**: δ 8.99 (dd, 1H, H2, $J_{2,3}$ = 7.1 Hz, $J_{2,4}$ = ~1 Hz), 8.58 (dd, 1H, H4, $J_{3,4}$ = 11.9 Hz), 8.19 (dd, 1H, H9, $J_{8,9}$ = 11 Hz, $J_{7,9}$ = ~1 Hz), 8.15–7.78 (m, 3H, H7, H3, H8), 7.32–7.09 (m, 9H, H1′, 8×Ar–H), 4.97 (m, 2H, CH_2–Ar), 2.33 (s, 3H, Me–Ar), 1.91 (d, 3H, H2′, $J_{1',2'}$ = 7.2 Hz). **16**: δ 8.98 (dd, 1H, H2, $J_{2,3}$ = 6 Hz, $J_{2,4}$ = ~1 Hz), 8.57 (dd, 1H, H4, $J_{3,4}$ = 11.4 Hz), 8.10 (d, 1H, H9, $J_{8,9}$ = 11 Hz), 8.01 (d, 1H, H7, $J_{7,8}$ = 11 Hz), 7.93–7.83 (m, 2H, H3, H8), 7.71–7.25 (m, 7H, H1′, 6×Ar–H), 6.85 (m, 2H, Ar–H), 5.19 (s, 2H, CH_2–Ar), 2.40 (s, 3H, Me–Ar), 1.78 (d, 3H, H2′, $J_{1',2'}$ = 7 Hz). **17**: δ 8.99 (dd, 1H, H2, $J_{2,3}$ = 7.4 Hz, $J_{2,4}$ = 1.5 Hz), 8.56 (dd, 1H, H4, $J_{3,4}$ = 9.2 Hz), 8.10 (dd, 1H, H9, $J_{8,9}$ = 8.7 Hz, $J_{7,9}$ = 1.2 Hz), 8.03 (dd, 1H, H7, $J_{7,8}$ = 7.8 Hz), 7.96 (br.s, 1H, H3), 7.74–7.69 (m, 2H, H8, Ar–H), 7.51–7.28 (m, 7H, H1′, 6×Ar–H), 6.98 (d, 1H, Ar–H, J = 8.4 Hz), 5.18 (br.s, 2H, CH_2–Ar), 2.27 (s, 3H, Me), 1.81 (d, 3H, H2′, $J_{1',2'}$ = 6.5 Hz). **18**: δ 9.00 (d, 1H, H2, $J_{2,3}$ = 11 Hz), 8.60 (d, 1H, H4, $J_{8,9}$ = 11 Hz), 8.25–7.97 (m, 4H, H9, H7, H3, H8), 7.61–7.10 (m, 5H, H1′, 4×Ar–H), 1.90 (d, 3H, H2′, $J_{1',2'}$ = 7 Hz). **19**: δ 9.00 (dd, 1H, H2, $J_{2,3}$ = 7.3 Hz, $J_{2,4}$ = ~1 Hz), 8.59 (dd, 1H, H4, J_{3-4} = 11.4 Hz), 8.21 (dd, 1H, H9, $J_{8,9}$ = 7.0 Hz, $J_{7,9}$ = ~1 Hz), 8.09–7.94 (m, 4H, H7, H3, H8, Ar–H), 7.82 (m, 1H, Ar–H), 7.52–7.41 (m, 2H, H1′, Ar–H), 7.25 (m, 1H, Ar–H), 1.95 (d, 3H, H2′, $J_{1',2'}$ = 6.4 Hz). **20**: δ 9.00 (dd, 1H, H2, $J_{2,3}$ = 7.7 Hz, $J_{2,4}$ = ~1 Hz), 8.59 (dd, 1H, H4, $J_{3,4}$ = 9.9 Hz), 8.23–7.96 (m, 6H, H9, H7, H3, H8, 2×Ar–H), 7.43 (q, 1H, H1′), 7.15 (m, 2H, Ar–H), 1.89 (d, 3H, H2′, $J_{1',2'}$ = 6.7 Hz). **22**: δ 9.01 (dd, 1H, H2, $J_{2,3}$ = 7.3 Hz, $J_{2,4}$ = 1.2 Hz), 8.61 (dd, 1H, H4, $J_{3,4}$ = 9.4 Hz), 8.24 (dd, 1H, H9, $J_{7,9}$ = 0.7 Hz, $J_{8,9}$ = 9.2 Hz), 8.14 (m, 1H, H7), 8.07 (m, 1H, H3), 8.02 (m, 1H, H8), 7.53–7.38 (m, 5H, 4×Ar–H, H1′), 1.90 (d, 3H, H2′, $J_{1',2'}$ = 6.8 Hz). **23**: δ 8.90 (dd, 1H, H2, $J_{2,3}$ = 7.3 Hz, $J_{2,4}$ = 1.5 Hz), 8.50 (dd, 1H, H4, $J_{3,4}$ = 8.8 Hz), 8.15 (dd, 1H, H7, $J_{7,8}$ = 8.7 Hz, $J_{7,9}$ = 1.5 Hz), 8.05–7.90 (m, 5H, H3, H8, H9, 2×Ar–H), 7.49 (m, 1H, Ar–H), 7.37 (q, 1H, H1′), 7,35 (t, 1H, Ar–H), 1.91 (d, 3H, H2′, $J_{1',2'}$ = 6.6 Hz). **26**: δ 8.99 (dd, 1H, H2, $J_{2,3}$ = 7.3 Hz, $J_{2,4}$ = 1.5 Hz), 8.59 (dd, 1H, H4, $J_{3,4}$ = 9.8 Hz), 8.21 (m, 1H, H9), 8.06 (m, 1H, H7), 8.05–7.90 (m, 3H, H3, H8, Ar–H), 7.81–7.60 (m, 3H, Ar–H), 7.47 (q, 1H, H1′), 1.90 (d, 3H, H2′, $J_{1',2'}$ = 6.4 Hz). **27**: ^{1}H NMR ($CDCl_3$): δ 9.00 (dd, 1H, H2, $J_{2,3}$ = 6.8 Hz, $J_{2,4}$ = 1.7 Hz), 8.65 (dd, 1H, H4, $J_{3,4}$ = 9.0 Hz), 8.57 (m, 1H, H7), 8.46 (dd, 1H, H2, $J_{2,3}$ = 9 Hz, $J_{2,4}$ = 1.5 Hz), 8.35 (dd, 1H, H4, $J_{3,4}$ = 7 Hz), 8.25 (dd, 1H, H9, $J_{8,9}$ = 7 Hz, $J_{7,9}$ = 1.5 Hz), 8.21 (m, 1H, H7), 8.14 (m, 1H, H2″), 8.03–8.09 (m, 3H, H3, H8, H6″), 8.00 (dd, 1H, H9, $J_{8,9}$ = 5 Hz, $J_{7,9}$ = ~1 Hz), 7.92–7.88 (m, 2H, H3, H8), 7.67 (q, 1H, H1′), 7.57 (m, 1H, H-4″), 7.49 (dq, 1H, H1′, J = 2.1 Hz), 7.43 (t, 1H, H5″, J = 7.7 Hz), 1.96 (d, 3H, H2′, $J_{1'2'}$ = 6.4 Hz), 1.91 (d, 3H, H2′, $J_{1'2'}$ = 6.8 Hz).