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An Efficient, Convenient Synthesis of Novel Medium-Sized 13*H*-Dibenzo[*d*,*h*][1,3,7]oxadiazecine-8,14-dione Macrolides as Anticipated Antineoplastic Agents

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Abstract—A series of novel medium-sized 13*H*-dibenzo[*d*,*h*][1,3,7]oxadiazecine-8,14-dione macrolides (**18**–**27**, **30**, **32**) were synthesized in an ongoing effort to develop new antineoplastic agents. The synthon 2-(2-aminobenzoylamino)-benzoic acid (**7**), for preparation of the target compounds, was prepared via the reaction of isatoic anhydride **5** and anthranilic acid **6**. Nine compounds (**18**–**20**, **24**–**27**, **30**, **32**) were subjected to National Cancer Institute (NCI) in vitro disease-oriented human cells screening panel assay. Among the compounds tested, 6-benzyl-13*H*-dibenzo[*d*,*h*][1,3,7]oxadiazecine-8,14-dione (**26**, NSC 721327), bearing the benzyl group at position 6, showed cytotoxic activity and subpanel selectivity against leukemia (CCRF-CEM), colon (HCC-2998), CNS (SNB-75) and melanoma (UACC-257) panels at $\log_{10}$ GI_{50} (M), compound concentration that inhibits 50% of cell growth, ranging from −4.08 to −4.59.

Introduction

Medium ring macrcyclic lactones, those having a ring size of 8 to 11,[1] are an extremely important class of compounds in organic chemistry as they are incorporated in an ever-increasing number of natural and synthetic products. Marked anti-inflammatory, antibacterial, antiviral and antitumor activities emerging by medium ring macrocyclic lactones combining with nitrogen substituents or nitrogen heterocycles have evoked interest in the search for useful leads in the development of new pharmaceutical agents.[2–7] The synthesis of these compounds was much more difficult by cyclization methods than other macrocyclic compounds (ring size $\geq$12). These difficulties are noticed since the formation of these cyclic compounds are disfavoured by entropy as well as enthalpy.[8] Development of an efficient method for preparation of medium-ring lactone compounds continues to be an important theme in synthetic organic chemistry. Generally these lactones are prepared: (1) from ω-halo or hydroxycarboxylic acids; (2) from cycloakanes by Baeyer-villiger oxidation; (3) by fusion-bond cleavage of bicyclic compounds; (4) from cycloalkanones di- and triperoxides; (5) by reverse Diekmann reaction; and (6) from acyclic esters.[9] ω-Hydroxycarboxylic acids are the most common starting materials because of their relative availability for large-ring lactone formation which can be achieved via direct esterification, transesterification, mixed anhydride, *N*-acylimidazolides, thiol esters, 2-chloropyridinum and 2-chlorobenzothiazolium salts and hydroxy activation.[9] One of the methods commonly used is the use of the appropriate hydroxy acids and trifluoroacetic anhydride in benzene solution at room temperature.[10] However, this procedure suffers limitations such as strong acidic conditions (CF_3COOH) employed and low yields which made this method not an attractive one. Encouraged by the fact that an increasing number of the macrocyclic lactones exhibited cytotoxic activity,[11–13] we report here the synthesis and antineoplastic activity of novel medium sized 13*H*-dibenzo[*d*,*h*][1,3,7]oxadiazecine-8,14-dione macrolides (**18**–**27**, **30**, **32**). In addition to our interest in this area to search for lead antitumor agents, an efficient, convenient and simple cyclization method for synthesis of the target compounds (**18**–**27**, **30**, **32**) was adopted.

Chemistry

In the present investigation, we prepared 12 new medium-sized nitrogen containing 13*H*-dibenzo[*d*,*h*][1,3,7]oxa-

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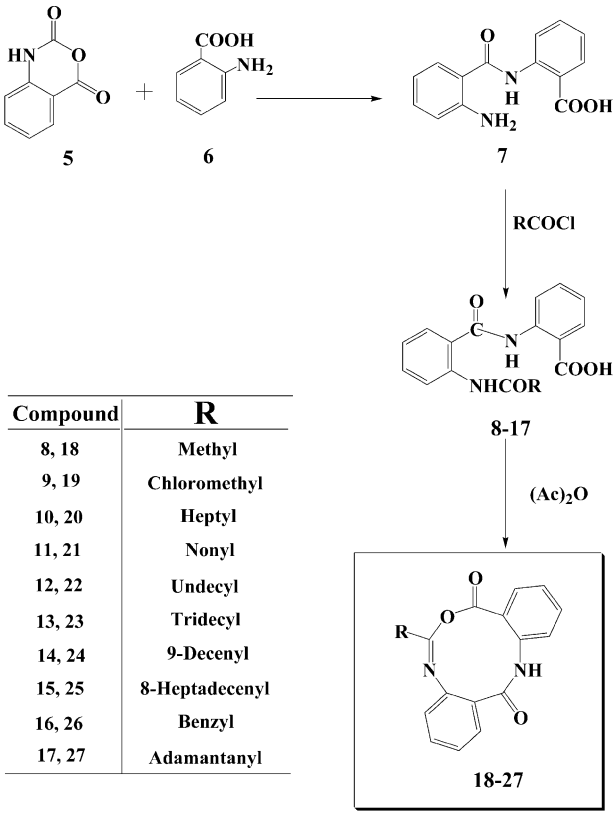
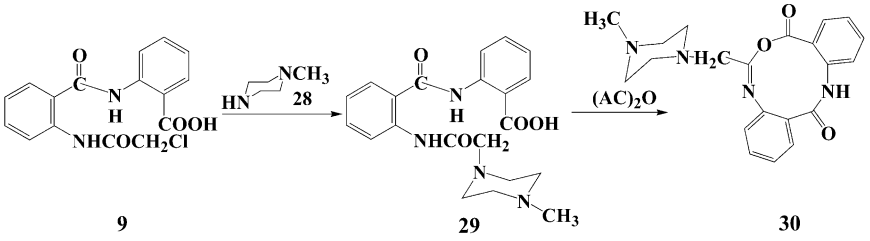
diazecine-8-14-dione macrolides (**18**–**27**, **30**, **32**). The routes for preparation of these compounds are illustrated in Schemes 1–3. Reaction of anthranilic acid **6** with phosgene leads to isatoic anhydride **5**. In contrast, the latter reacted with **6** to afford the synthon 2-(2-aminobenzoylamino)-benzoic acid (**7**).[14,15] Generally, nucleophiles react with **5** at one of the two carbonyl groups at the C-2 or C-4 position. The C-4 position, which resembles an anhydide, is more reactive than C-2 which acts like a carbamate in reactivity.[16] The structure of **7** was assigned on the bases of its EI-MS, IR and NMR spectral data. The EI-MS spectra showed a molecular ion peak at m/z 256 $[M]^+$, which was consistent with its molecular formula. Absorption bands at 3400–3300 cm^{-1} (NH_2, OH), 1695 (acid C=O) and 1650 (amide C=O) were seen in the IR spectrum. In the ^1H NMR spectrum, signals for aromatic protons appeared at δ 8.66 ~ 6.64 ppm and signals for NH and NH_2 protons appeared at δ 11.94 and 3.45 ppm. The structure of **7** was further confirmed by ^{13}C NMR data that revealed, in addition to the aromatic protons, signals at δ 170.0 and 167.4 due to the carbonyl carbon of COOH and CONH, respectively. Compound **7** reacted with acyl chloride in dioxane to give the intermediates **7**–**18**. The structures of **7**–**18** were confirmed by spectroscopic means including IR, NMR and EI-MS (Tables 1–3). A novel nitogen containing lactam lactone type structure, sandwiched between two benzene rings, (**18**–**27**) was obtained by intramolecular cyclization of **8**–**17**. As is clear from the introductory part, esterification of hydroxy acids is the most common method used for preparation of medium ring lactones. Different methods were utilized for such esterification and the use of trifluroacetic anhydride in benzene solution is the famous one.[10] Since this method employed strong acidic conditions and usually low yields were obtained, we performed esterification of the intermediates **8**–**17** to afford the target compounds **18**–**27**, using acetic anhydride for 15 min. A rapid esterification process of hydroxy acids to prepare medium ring lactones is necessary to avoid the formation of polymers and overcome the undesirable entropy factors. The use of acetic anhydride was mild and a rapid esterification method successfully used for the synthesis of the target lactones (**18**–**27**) in good yields. On the basis of careful inspection of the spectroscopic data of compounds **18**–**27**, their structures were determined. The EI-MS spectra of these compounds revealed molecular ion peaks with 18 mass units less than that of **8**–**17** precursors, respectively, indicating that **18**–**27** were dehydrated products of **8**–**17**. These molecular ion peaks were consistent with their respective molecular formulas.

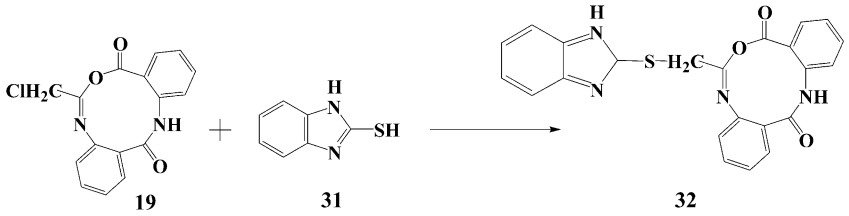


Compound	R
8, 18	Methyl
9, 19	Chloromethyl
10, 20	Heptyl
11, 21	Nonyl
12, 22	Undecyl
13, 23	Tridecyl
14, 24	9-Decenyl
15, 25	8-Heptadecenyl
16, 26	Benzyl
17, 27	Adamantanyl

Scheme 1.



Scheme 2.



Scheme 3.

Table 1. Physical and IR data of 2-(2-acylaminobenzoylamino)-benzoic acid derivatives (**8**–**17**, **29**)

Compound[a]	Mp (°C)	Yield (%)	Solvent[b]	Molecular formula	IR KBr (cm^{-1})
8	>300	95	A	$C_{16}H_{14}N_2O_4$	3200 (NH), 1695, 1675, 1665 (C=O), 1610, 1200
9	>300	90	A	$C_{16}H_{13}N_2O_4$	3200 (NH), 1675, 1660, 1640 (C=O), 1610, 1230
10	>300	92	B	$C_{22}H_{26}N_2O_4$	3200 (NH), 1690, 1660, 1640 (C=O), 1610, 1240
11	>300	90	B	$C_{24}H_{30}N_2O_4$	3200 (NH), 1692, 1660, 1640 (C=O), 1610, 1240
12	>300	88	B	$C_{26}H_{34}N_2O_4$	3200 (NH), 1690, 1660, 1640 (C=O), 1610, 1240
13	>300	87	B	$C_{28}H_{38}N_2O_4$	3200 (NH), 1690, 1660, 1640 (C=O), 1610, 1250
14	>300	82	B	$C_{25}H_{30}N_2O_4$	3200 (NH), 1690, 1660, 1645 (C=O), 1610, 1240
15	223–225	80	B	$C_{32}H_{44}N_2O_4$	3200 (NH), 1690, 1665, 1640 (C=O), 1610, 1225
16	>300	96	D	$C_{22}H_{18}N_2O_4$	3200 (NH), 1695, 1660, 1618 (C=O), 1610, 1240
17	>300	85	C	$C_{25}H_{26}N_2O_4$	3220 (NH), 1680, 1660, 1650 (C=O), 1610, 1230
29	>300	78	C	$C_{21}H_{24}N_4O_4$	3200 (NH), 1690, 1665, 1640 (C=O), 1610, 1245

[a]The mass spectrum revealed a proper molecular ion $[M]^+$ for all compounds.
[b]Solvent of crystallization: A, $CHCl_3$–MeOH; B, $CHCl_3$–acetone; C, aqueous ethanol; D, $CHCl_3$.

Table 2. ^1H NMR data of 2-(2-acylaminobenzoylamino)-benzoic acid derivatives (**8**–**17**, **29**)

Compound[a]	–C(O)–NH—	–NH–C(O)–R	Ar–H	Others
8	11.84	10.46	6.85–8.07 (8H)	2.01 (3H, s, CH_3)
9	11.97	11.23	7.23–8.59 (8H)	4.39 (2H, s, $-CH_2Cl$)
10	11.83	10.48	7.20–8.61 (8H)	2.30 (2H, t, $-CH_2-$), 1.54 (2H, p, $-CH_2-$), 1.22 (8H, m, 4×$-CH_2-$), 0.82 (3H, t, $-CH_3$)
11	11.86	10.51	7.18–8.61 (8H)	2.30 (2H, t, $-CH_2-$), 1.57 (2H, p, $-CH_2-$), 1.21 (12H, m, 6×$-CH_2-$), 0.84 (3H, t, $-CH_3$)
12	11.85	10.50	7.20–8.61 (8H)	2.30 (2H, t, $-CH_2-$), 1.56 (2H, p, $-CH_2-$), 1.21 (16H, m, 8×$-CH_2-$), 0.84 (3H, t, $-CH_3$)
13	11.86	10.51	7.19–8.61 (8H)	2.30 (2H, t, $-CH_2-$), 1.53 (2H, p, $-CH_2-$), 1.21 (20H, m, 10×$-CH_2-$), 0.84 (3H, t, $-CH_3$)
14	11.85	10.49	7.20–8.61 (8H)	5.76 (1H, m, $-CH-CH_2$), 4.93 (2H, m, $-CH-CH_2$), 2.30 (2H, t, $-CH_2-$), 1.97 (2H, q, $-CH_2-$), 1.52 (2H, p, $-CH_2-$), 1.25 (10H, m, 5×$-CH_2-$)
15	11.87	10.51	7.20–8.62 (8H)	5.31 (2H, m, $-CH-$), 2.30 (2H, t, $-CH_2-$), 1.94 (4H, m, 2×$-CH_2-$), 1.52 (2H, p, $-CH_2-$), 1.22 (20H, m, 10×$-CH_2-$), 0.83 (3H, t, $-CH_3$)
16	11.83	10.61	7.21–8.56 (13H)	3.69 (2H, s, $-CH_2-$)
17	11.92	10.95	6.83–8.62 (8H)	1.64–2.01 (15H, adamantanyl moiety)
29	12.02	11.73	7.01–8.72 (8H)	3.31 (2H, s, $-CH_2-$), 2.69 (4H, m, 2×$-CH_2-$), 2.40 (4H, m, 2×$-CH_2-$), 2.01 (3H, s, N–CH_3)

[a]All compounds measured in DMSO.

Table 3. ^{13}C NMR data of 2-(2-acylaminobenzoylamino)-benzoic acid derivatives (**8**–**17**, **29**)

Compound[a]	–COOH	–C(O)–NH–	R–C(O)–NH–	Ar–C	Others
8	169.9	168.3	166.7	116.6, 120.0, 120.5, 122.8, 123.2, 123.7, 127.4, 131.2, 132.8, 134.1, 137.5, 141.0	24.2 (CH_3)
9	169.7	166.3	165.0	117.6, 120.7, 122.0, 123.5, 124.0, 124.4, 127.9, 131.2, 132.4, 134.1, 137.3, 140.3	43.3 ($-CH_2Cl$)
10	171.2	169.5	166.4	117.2, 120.4, 122.3, 123.1, 123.6, 124.8, 127.8, 131.1, 132.0, 134.0, 137.7, 140.6	13.8, 21.9, 24.9, 28.3, 28.4, 31.0, 36.8 (6×CH_2, CH3)
11	171.1	169.6	166.4	117.1, 120.4, 122.3, 123.1, 123.6, 124.6, 127.8, 131.1, 132.0, 134.0, 137.7, 140.6	13.8, 22.0, 24.8, 28.4, 28.6, 28.7, 28.7, 31.2, 36.8 (8×CH_2, CH3)
12	171.2	169.6	166.4	117.1, 120.4, 122.3, 123.1, 123.6, 124.6, 127.8, 131.0, 132.0, 133.9, 137.7, 140.6	13.8, 22.0, 24.8, 28.4, 28.6, 28.6, 28.7, 28.9, 28.9, 31.2, 36.8 (10×CH_2, CH3)
13	171.2	169.6	166.4	117.2, 120.4, 122.3, 123.1, 123.6, 124.7, 127.8, 131.1, 132.0, 134.0, 137.8, 140.6	13.9, 22.0, 24.9, 28.4, 28.6, 28.7, 28.8, 28.9, 28.9, 29.0, 29.0, 31.2, 36.8 (12×CH_2, CH3)
14	171.2	169.6	166.4	117.2, 120.4, 122.4, 123.2, 123.7, 124.8, 127.9, 131.1, 132.0, 134.0, 137.6, 140.6	24.9, 28.2, 28.4, 28.4, 28.6, 28.7, 33.2, 36.8 (8×CH_2), 114.6 (CH_2=CH–), 138.8 (CH_2=CH–)
15	171.1	169.6	166.4	117.1, 120.4, 122.3, 123.1, 123.6, 124.6, 127.8, 131.1, 132.0, 134.0, 137.6, 140.6	13.9, 22.1, 24.9, 26.5, 28.4, 28.5, 28.6, 28.8, 28.9, 28.9, 29.0, 31.2, 31.9, 33.6, 36.8 (14×CH_2, CH_3), 129.6, 130.0 (–CH=CH–)
16	169.5	169.2	166.2	117.2, 120.5, 122.2, 123.2, 123.8, 124.7, 126.6, 127.7, 128.2, 128.6, 131.0, 132.0, 134.0, 135.1, 137.6, 140.4	43.7 ($-CH_2$–Ph)
17	169.9	167.0	166.7	116.6, 120.0, 120.9, 122.8, 123.1, 123.6, 127.4, 131.2, 132.7, 134.1, 137.5, 141.0	77.9, 75.8, 41.3, 38.6, 35.9, 27.5 (adamantanyl carbons)
29	170.4	168.7	166.4	119.0, 120.3, 122.3, 122.4, 122.7, 123.1, 127.7, 131.4, 131.6, 132.2, 138.6, 140.4	43.9 (N–CH_3), 52.6, 52.8 (piperazinyl CH_2), 53.7 ($-CH_2-$)

[a]All compounds measured in DMSO.

Evidence of lactone formation in **18**–**27** emerged from the presence of absorption bands in their IR spectra at 1770∼1785 cm^{-1}, assigned to ester C=O. On the other hand, absorption bands at 1618∼1665 cm^{-1}, assigned to the exocyclic amide carbonyl in the IR spectra of **8**–**17**, were not seen, while all other signals remain significantly unaffected (Table 4). The ^1H NMR spectra of **18**–**27** were comparable with that of **8**–**17**, except for the absence of signals at δ 10.46∼11.73 ppm assigned to –NH–C(O)–R protons (Table 5). Another proof for intramolecular cyclization and lactone formation in **18**–**27** was picked up from ^{13}C NMR spectra (Table 6). The disappearance of signals at δ 169.5∼171.2 and 165.0∼166.7 ppm, assigned to carbonyl groups of COOH and –C(O)–NH–, respectively, in **8**–**17**, were noticed. The appearance of signals at δ 158.1∼158.7 and 156.3∼157.7 ppm, assigned to C=O of ester and C=N, respectively, in **18**–**27**, were observed. Based on the above findings with the aid of elemental analyses that showed satisfactory results within the accepted experimental errors, the structures of **18**–**27** were established.

Trials to react 6-(chloromethyl)-13*H*-dibenzo[*d*,*h*][1,3,7] oxadiazecine-8,14-dione **19** with *N*-methyl-piperazine **28** were unsuccessful. Even at room temperature in the presence of **28** or amines such as cyclohexyl amine and morpholine, the 10-membered ring lactone structure of **19** was opened. For this reason, compound **30** was synthesized via cyclization of the intermediate **29**. On the other hand, compound **19** reacts with 2-mercaptobenzimidazole **31** to afford **32**. The structures of **30** and **32** were assigned depending on the bases of spectral data and elemental analyses (Tables 4–6).

Pharmacological Results and Discussion

As a primary screening, nine compounds (**18**–**20**, **24**–**27**, **30**, **32**) out of the prepared compounds were selected by

Table 4. Physical and IR data of 6-(substituted)-13*H*-dibenzo[*d*,*h*][1,3,7]oxadiazecine-8,14-dione derivatives (**18**–**27**, **30**, **32**)

Compound[a]	Mp (°C)	Yield (%)	Solvent[b]	Molecular formula[c]	IR KBr (cm^{-1})
18	180–181	92	A	$C_{16}H_{12}N_2O_3$	3200 (NH), 1780, 1710 (C=O), 1610, 1250
19	156–158	89	A	$C_{16}H_{11}N_2O_3$	3200 (NH), 1770, 1695 (C=O), 1610, 1220
20	106–107	85	B	$C_{22}H_{24}N_2O_3$	3200 (NH), 1770, 1698 (C=O), 1610, 1230
21	139–140	88	B	$C_{24}H_{28}N_2O_3$	3200 (NH), 1775, 1698 (C=O), 1610, 1230
22	104–105	84	B	$C_{26}H_{32}N_2O_3$	3200 (NH), 1770, 1698 (C=O), 1610, 1230
23	98–99	81	B	$C_{28}H_{36}N_2O_3$	3200 (NH), 1770, 1700 (C=O), 1610, 1240
24	243–244	90	B	$C_{25}H_{28}N_2O_3$	3200 (NH), 1772, 1698 (C=O), 1610, 1230
25	58–60	78	B	$C_{32}H_{42}N_2O_3$	3200 (NH), 1770, 1695 (C=O), 1610, 1210
26	192–193	93	D	$C_{22}H_{16}N_2O_3$	3200 (NH), 1785, 1700 (C=O), 1610, 1220
27	178–180	74	C	$C_{25}H_{24}N_2O_3$	3220 (NH), 1775, 1695 (C=O), 1610, 1250
30	51–52	83	C	$C_{21}H_{22}N_4O_3$	3200 (NH), 1775, 1698 (C=O), 1610, 1230
32	186–187	70	D	$C_{23}H_{16}N_4O_3S$	3200 (NH), 1778, 1680 (C=O), 1610, 1250

[a]The mass spectrum revealed a proper molecular ion $[M]^+$ for all compounds.
[b]Solvent of crystallization: A, aqueous DMF; B, ether–$CHCl_3$; C, ethanol–$CHCl_3$; D, ethanol.
[c]Satisfactory microanalysis were obtained (±0.4%) for C, H and N.

Table 5. ^1H NMR data of 6-(substituted)-13*H*-dibenzo[*d*,*h*][1,3,7]oxadiazecine-8,14-dione derivatives (**18**–**27**, **30**, **32**)

Compound	–C(O)—NH—	Ar–H	Others
18[a]	11.65	7.26–8.40 (8H)	2.22 (3H, s, CH_3)
19[a]	12.37	7.33–8.56 (8H)	4.54 (2H, s, –CH_2Cl)
20[a]	12.62	7.24–8.43 (8H)	2.47 (2H, t, –CH_2–), 1.67 (2H, p, –CH_2–), 1.28 (8H, m, 4×–CH_2–), 0.83 (3H, t, –CH_3)
21[a]	11.94	7.21–8.59 (8H)	2.49 (2H, t, –CH_2–), 1.73 (2H, p, –CH_2–), 1.32 (12H, m, 6×–CH_2–), 0.84 (3H, t, –CH_3)
22[b]	12.29	7.16–8.84 (8H)	2.54 (2H, t, –CH_2–), 1.85 (2H, p, –CH_2–), 1.36 (16H, m, 8×–CH_2–), 0.86 (3H, t, –CH_3)
23[b]	12.29	7.16–8.84 (8H)	2.54 (2H, t, –CH_2–), 1.85 (2H, p, –CH_2–), 1.36 (20H, m, 10×–CH_2–), 0.87 (3H, t, –CH_3)
24[b]	12.28	7.16–8.84 (8H)	5.78 (1H, m, –CH–CH_2), 4.95 (2H, m, –CH–CH_2), 2.54 (2H, t, –CH_2–), 2.02 (2H, q, –CH_2–), 1.84 (2H, p, –CH_2–), 1.36 (10H, m, 5×–CH_2–)
25[b]	12.29	7.16–8.83 (8H)	5.34 (2H, m, –CH–), 2.54 (2H, t, –CH_2–), 2.00 (4H, m, 2×–CH_2–), 1.84 (2H, p, –CH_2–), 1.34 (20H, m, 10×–CH_2–), 0.87 (3H, t, –CH_3)
26[a]	11.77	7.23–8.44 (13H)	3.85 (2H, s, –CH_2–)
27[c]	11.62	7.24–8.68 (8H)	1.75–2.20 (15H, adamantanyl moiety)
30[b]	12.36	7.18–8.82 (8H)	3.34 (2H, s, –CH_2–), 2.72 (4H, m, 2×–CH_2–), 2.47 (4H, m, 2×–CH_2–), 2.22 (3H, s, N–CH_3)
32[a]	12.22	7.27–8.43 (12H)	12.52 (1H, br s, NH), 4.58 (2H, s, S–CH_2–)

[a]DMSO.
[b]$CDCl_3$.
[c]$CDCl_3$–DMSO.

NCI to be screened for their in vitro antitumor activity according to the standard protocol of NCI (Bethesda, MD, USA).[17,18] For the past 10 years, the Development Therapeutic Program (DTP), Division of Cancer Treatment and Diagnosis (DCTD), NCI has used an in vitro model consisting of 60 human tumor cell lines as the primary anticancer screen. An analysis of the data indicated that approximately 95% of the actives from the 60 cell lines screen can be identified using only three cell lines. For this reason, the DTP has now begun using, as its primary anticancer assay, a three-cell lines panel consisting of the MCF7 (Breast), NCI-H460 (Lung), and SF-268 (CNS). In the current protocol, each cell line is inoculated and pre-incubated on a microtiter plate. Test agents are then added at a single concentration and the culture incubated for 48 h. End-point determinations are made with alamar blue. Results for each test agent are reported as the percent of growth of the treated cells when compared to the untreated control cells. Compounds which reduce the growth of any one of the cell lines to approximately 32% or less are passed on for evaluation in the full panel of 60 cell lines over a 5-log dose range. In these three-cell lines, one dose primary anticancer assay, 6-benzyl-13*H*-dibenzo[*d*,*h*][1,3,7]oxadiazecine-8–14-dione (**26**, NSC 721327) passed on for evaluation against 60 human cell lines. Leukemia, lung, colon, central nervous system, melanoma, ovary, renal, prostate and breast cancer were the nine clinically isolated cancer subpanels representing 60 -human tumor cell lines. Compound 26, bearing the benzyl group at position 6, displayed cytotoxic activity and subpanel selectivity against leukemia (CCRF-CEM), colon (HCC-2998), CNS (SNB-75) and melanoma (UACC-257) panels at $\log_{10}$ GI_{50} (M) ranging from −4.08 to −4.59.

Conclusion

The present paper deals with the design and synthesis of novel series of medium-sized nitrogen containing 13*H*-dibenzo[*d*,*h*][1,3,7]oxadiazecine-8,14-dione macrolides (**18**–**27**, **30**, **32**) in an attempt to obtain a novel antitumor structure. The new compounds (**18**–**27**, **30**, **32**) described here represent a novel class of compounds incorporated in a 10-membered ring lactone lactam type structure. The results obtained could not confirm our anticipation that they would stimulate further structural modifications of this class of compounds. On the other hand, exploration of the antibacterial and antiviral activities of these derivatives will be a subject of further investigations.

Experimental

Melting points were determined on a micromelting point apparatus (L-272, Yanaco, Kyoto, Japan) and are uncorrected. IR spectra were performed with a JASCO FT/IR-230 infrared spectrophotometer. Thin layer chromatography was carried out on pre-coated Silica gel 60 F_{254} plates and reversed-phase RP-18 F_{254} S plates (0.25 mm thickness, Merck, Darmstadt, Germany) and spots were detected under UV light or after spraying with anisaldehyde–H_2SO_4 reagent followed by heating. Silica gel 60 (70–230 mesh, Merck, Darmstadt, Germany) was used for column chromatography. ^{1}H and ^{13}C NMR spectra were measured with a JEOL-JNM-GX 400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) spectrophotometer and chemical shifts were given in δ ppm relative to tetramethylsilane (TMS). Electron impact

Table 6. ^{13}C NMR data of 6-(substituted)-13*H*-dibenzo[*d*,*h*][1,3,7]oxadiazecine-8,14-dione derivatives (**18**–**27**, **30**, **32**)

Compound	–C(O)–NH–	–C(O)–O–	–C=N–	Ar–C	Others
18[a]	168.5	158.3	156.8	116.7, 117.0, 120.9, 123.1, 126.6, 128.1, 128.9, 129.4, 133.2, 137.0, 139.2, 145.4	26.7 (CH_3)
19[a]	168.5	158.1	156.3	116.8, 116.9, 120.6, 123.9, 126.3, 128.1, 129.0, 129.5, 133.5, 137.0, 138.5, 145.0	43.8 (CH_2Cl)
20[a]	171.4	158.2	156.8	116.7, 117.0, 120.8, 123.0, 126.3, 128.0, 128.9, 129.4, 133.2, 137.0, 139.1, 145.5	13.8, 21.9, 25.0, 28.4, 28.5, 31.0, 37.4 (6×CH_2, CH3)
21[a]	171.2	158.7	156.7	115.6, 116.4, 120.3, 122.5, 126.0, 128.0, 128.6, 129.0, 133.1, 136.6, 139.6, 145.8	13.7, 21.9, 25.0, 28.5, 28.5, 28.7, 28.7, 31.1, 37.7 (8×CH_2, CH3)
22[b]	171.2	158.2	156.6	114.4, 116.8, 120.6, 122.7, 126.1, 128.8, 129.0, 129.5, 134.2, 136.8, 140.8, 145.4	14.1, 22.6, 25.8, 29.3, 29.4, 29.4, 29.5, 29.6, 29.6, 31.9, 39.1 (10×CH_2, CH3)
23[b]	171.2	158.2	156.6	114.4, 116.8, 120.6, 122.7, 126.1, 128.8, 129.0, 129.5, 134.2, 136.8, 140.8, 145.4	14.1, 22.7, 25.8, 29.3, 29.4, 29.4, 29.5, 29.6, 29.6, 29.6, 29.7, 31.9, 39.1 (12×CH_2, CH3)
24[b]	171.1	158.2	157.6	114.4, 116.8, 120.6, 122.7, 126.1, 128.8, 129.0, 129.5, 134.2, 136.8, 140.8, 145.4	25.7, 28.8, 29.1, 29.3, 29.3, 29.3, 33.7, 39.1 (8×CH_2), 114.2 (CH_2=CH–), 139.1 (CH_2=CH–)
25[b]	171.9	158.3	157.6	114.5, 116.8, 120.7, 122.8, 126.1, 128.9, 129.1, 129.5, 134.3, 136.9, 140.8, 145.4	14.1, 22.7, 25.8, 27.2, 27.3, 29.0, 29.2, 29.4, 29.4, 29.5, 29.7, 29.7, 31.9, 32.6, 39.1 (14×CH_2, CH_3), 130.0, 130.3 (–CH=CH–)
26[a]	169.4	158.2	156.6	116.7, 117.1, 120.9, 122.2, 126.6, 126.7, 127.9, 128.4, 128.9, 129.2, 129.4,133.2, 135.2, 136.8, 139.0, 145.1	44.3 (–CH_2–Ph)
27[c]	176.2	157.6	156.7	115.7, 116.5, 120.4, 122.3, 125.2, 128.2, 128.6, 129.1, 133.1, 136.7, 139.9, 145.0	78.7, 76.4, 41.7, 38.8, 36.0, 27.5 (adamantanyl carbons)
30[b]	169.5	158.5	157.0	116.1, 116.8, 121.4, 123.2, 127.2, 128.8, 128.9, 129.6, 133.8, 136.5, 139.7, 145.7	45.8 (N–CH_3), 53.3, 54.1 (piperazinyl CH_2), 64.0 (–CH_2–)
32[a]	166.2	158.2	156.3	113.5, 116.7, 117.4, 121.1, 122.3, 123.3, 123.8, 126.9, 127.9, 129.0, 129.5, 133.3, 136.9, 138.5, 145.1, 149.3	37.0 (CH_2S)

[a]DMSO.
[b]$CDCl_3$.
[c]$CDCl_3$–DMSO.

(EI) mass spectra were measured with a JEOL GC-mate spectrometer at an ionization voltage of 70 eV. Elemental analyses were performed at the microanalytical center of Toyama Medical and Pharmaceutical University. All chemicals used were of analytical grade.

Synthesis of 2-(2-aminobenzoylamino)-benzoic acid (7).[14,15] A suspension of **5** (2.5 g, 0.015 mol) and **6** (2.3 g, 0.017 mol) in water (50 mL) was refluxed for 1 h and then cooled. The separated crystals were filtered off, washed with water and crystallized from $CHCl_3$–ethanol to afford **7**, yield (3.1 g, 79%) as pale yellow crystals, mp $>300\,^{\circ}C$. IR (KBr) $\nu\ cm^{-1}$: 3400–3300 (NH_2, OH), 3200 (NH), 1695 (acid C=O), 1650 (amide C=O). ^1H NMR (DMSO-d_6) δ: 11.94 (1H, s, NH), 8.66∼6.64 (8H, Ar–H) and 3.45 (2H, br S, NH_2). ^{13}C NMR (DMSO-d_6) δ: 170.0 (COOH), 167.4 (CONH), 150.5, 141.4, 134.2, 132.8, 131.2, 127.3, 122.5, 119.9, 117.1, 116.3, 115.2 and 114.0 (aromatic carbons). EI-MS m/z 256 $[M]^+$, 238 $[M-H_2O]^+$, 119, 92 and 65.

General method for synthesis of compounds 8–17

To an ice cooled solution of **7** (1 equiv mol) in dioxane, a solution of the appropriate acid chloride (1 equiv mol) in dioxane was added drop wise. The reaction mixture was stirred overnight at room temperature. The product after addition of water was filtered off, washed with water and crystallized from the proper solvents. Yields and physical constants are listed in Table 1. ^1H and ^{13}C NMR data are presented in Tables 2 and 3, respectively.

Synthesis of 2-{2-[2-(4-methyl-piperazin-1-yl)-acetyl-amino]-benzoylamino}-benzoic acid (29). *N*-Methyl piperazine (**28**, 0.3 g, 3 mmol) was added to a solution of **9** (0.4 g, 1.2 mmol) in DMF (3 mL) and the reaction mixture was refluxed for 1.5 h in an oil bath. The precipitate formed after addition of ice water was filtered, washed with water and then ether. The residue was purified by SiO_2 gel column chromatography using $CHCl_3$/MeOH as eluent. Yield and physical constants are listed in Table 1. ^1H and ^{13}C NMR data are presented in Tables 2 and 3, respectively.

General method for synthesis of compounds 18–27, 30

A solution of the appropriate benzoic acid derivative **8**–**17**, **29** (0.3 g) in acteic anhydride (5 mL) was refluxed for 15 min. After cooling to room temperature, the products precipitated were filtered and crystallized from the proper solvents. Yields and physical constants are listed in Table 4. ^1H and ^{13}C NMR data are presented in Tables 5 and 6, respectively.

Synthesis of 6-(1H-benzimidazol-2-ylthiomethyl)-13*H*-dibenzo[*d*,*h*] [1,3,7] oxadiazecine-8,14-diones (32). To a stirred solution of **19** (0.4 g, 1.27 mmol) in benzene (5 mL), 2-mercaptobenzimidazol **31** (0.19 g, 1.27 mmol) and pyridine (0.1 mL) was added. The reaction mixture was refluxed for 5 h and the precipitate formed after cooling was filtered off and crystallized from the proper solvent. Yield and physical constants are listed in Table 4. ^1H and ^{13}C NMR data are presented in Tables 5 and 6, respectively.

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