Bioorganic & Medicinal Chemistry 18 (2010) 2029–2036

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Biomimetic synthesis and anti-HIV activity of dimeric phloroglucinols

Siddheshwar K. Chauthe [a], Sandip B. Bharate [a], Sudeep Sabde [b], Debashis Mitra [b,*], Kamlesh K. Bhutani [a], Inder P. Singh [a,*]

[a] Department of Natural Products, National Institute of Pharmaceutical Education and Research (NIPER)[†], Sector-67, S.A.S. Nagar, Punjab 160 062, India
[b] National Center for Cell Science (NCCS), Pune University Campus, Ganeshkhind, Pune, Maharashtra 411 007, India

ARTICLE INFO

Article history:
Received 3 November 2009
Revised 8 January 2010
Accepted 9 January 2010
Available online 15 January 2010

Keywords:
Phloroglucinol
Anti-HIV activity
CEM-GFP cells
Antivirals

ABSTRACT

Plants are an important source of a variety of bioactive compounds with different modes of action. Anti-HIV agents from plant sources can be useful in developing novel therapies for inhibiting HIV infection. Based on the reported anti-HIV activity of plant derived phloroglucinols, several new dimeric phloroglucinols were synthesized in the present study by varying substitution on aromatic ring and at methylene bridge. Some of the synthesized compounds have shown good HIV inhibitory activity in a human CD4+ T cell line (CEM-GFP) infected with HIV-1 $NL_{4.3}$ virus isolate. Structure–activity studies indicate that phenyl, 4-benzyloxy-1-phenyl and cyclohexyl substitution at methylene bridge gave compounds with better anti-HIV activity. Compounds **22** and **24** showed highest anti-HIV activity with an IC_{50} of 0.28 μM and 2.71 μM, respectively, former was more active than the positive standard AZT in cell based assay.

1. Introduction

Acquired immunodeficiency syndrome (AIDS) is a clinical syndrome caused by the infection with human immunodeficiency virus-1 (HIV-1), which causes profound immunosuppression. It has been a serious, life-threatening health problem since the first case was identified in 1981 and is the most quickly spreading disease of the century.[1] In 2008, an estimated 33.4 million people lived with the disease worldwide, 2.7 million people were newly infected with the deadly virus and it killed an estimated 2.0 million people, including 280,000 children. In the last 25 years, HIV/AIDS has claimed 25 million lives worldwide.[2] Currently used HAART therapy comprises of drugs that are either nucleoside/nucleotide reverse transcriptase inhibitors or non-nucleoside reverse transcriptase inhibitor or protease inhibitors or supplemented with the addition of a fusion inhibitor.[3] This present therapy is able to control the viral replication but fails to eradicate virus completely from body. Recently, US FDA approved Maraviroc (CCR5 blocker) and Raltegravir (HIV integrase inhibitor) for the treatment of HIV-1 infection in combination with other antiretroviral agents.[4] Most of the clinically used anti-HIV agents are nucleosides and their toxicity, adverse effects and drug resistance limit their use. Hence, there is an urgent need for newer anti-HIV agents with novel mechanism of action.

Donglei et al. have elaborately reviewed anti-HIV agents from natural resources belonging to several classes including terpenoids, coumarins, alkaloids, polyphenols, tannins and flavonoids.[5] Earlier, we reviewed natural products exhibiting anti-HIV activity.[6] Anti-HIV agents from these classes exhibit unique mechanism of action pertaining to their physicochemical properties. These natural product derived compounds can be used in conjunction with existing anti-HIV agents and thus possibility of drug resistance could be minimized in novel formulations both for therapeutic and preventive use. Some of the natural products such as calanolides,[7a] calceolarioside B,[7b] macrocarpals,[7c] betulinic acid,[7d] and chicoric acid[7e] have exhibited high anti-HIV activity. Calanolide A and a betulinic acid derivative are in Phase II and IIb of clinical trials.[7f] Naturally occurring phloroglucinol compounds have shown a diverse range of biological activities including anti-HIV activity.[8] These compounds mainly occur in the genera *Dryopteris, Aspidium*, *Myrtus*, *Mallotus*, *Hypericum*, *Eucalyptus* and *Helichrysum*.[8] Dimeric phloroglucinols comprise compounds having two phloroglucinol units joined either through a methylene linkage or by the formation of a chroman ring. Dimeric phloroglucinol mallotojaponin (**1**) isolated from *Mallotus japonicus* showed 90% inhibition of HIV-RTase activity at concentration of 25 μg/mL.[9] Phlorotannin 6,6′-bieckol (**2**) obtained from *Ecklonia cava* showed inhibition of HIV-1 induced syncytia formation (EC_{50} 1.72 μM), lytic effects (EC_{50} 1.23 μM), and viral p24 antigen production (EC_{50} 1.26 μM).[10] Macrocarpals A–E, which are phloroglucinol terpene adducts showed HIV-RTase inhibitory activity, macrocarpal-B (**3**) was the most active with an IC_{50} = 5.3 μM.[11] Polyisoprenylated phloroglucinol derivative laxifloranone (**4**) showed inhibition of cytopathic effect of in vitro HIV infection (EC_{50} = 0.62 μg/mL and

* Corresponding authors. Tel.: +91 2025708151 (D.M.); +91 172214683x2144 (I.P.S.).
E-mail addresses: dmitra@nccs.res.in (D. Mitra), ipsingh@niper.ac.in, ipsingh67@yahoo.com (I.P. Singh).
† NIPER Communication Number-465.

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.01.023

IC_{50} = 6.6 μg/mL),[12] and phloroglucinol-α-pyrone derivative arzanol (**5**) inhibited HIV-1 replication in T cells (IC_{50} = 5 μM).[13] Structures of these anti-HIV phloroglucinols are shown in Figure 1. Biogenetically dimeric phloroglucinols can be proposed to be formed by joining of two monomeric units via methylene linkage as depicted in Figure 2. Methionine is presumed to be a source for methylene unit.[14]

The objective of the present study was to find new anti-HIV compounds based on anti-HIV activity of phloroglucinol derivatives and as a part of our continuing efforts to synthesize naturally occurring phloroglucinol compounds and their analogues to explore their biological potential.[15–17] Several dimers containing phloroglucinol moiety were synthesized by changing length of acyl functionality and varying substitution at methylene bridge. All the synthesized compounds have been tested for their potential to inhibit the virus in HIV-1 infected T cell line and the active compounds were further evaluated for reverse transcriptase inhibitory activity.

2. Results and discussion

2.1. Synthesis of dimeric phloroglucinols

Based on the biogenetic pathway,[14] our synthetic strategy involved initial synthesis of monomeric units which can be further dimerized in a key biomimetic step as depicted in Scheme 1. The key monomeric precursors, **7–9** were synthesized from phloroglucinol (**6**). The Friedel–Craft acylation of phloroglucinol (**6**) using different acyl chlorides resulted in formation of mono- (**10–12**) and diacetyl phloroglucinols (**7–9**) in 60% and 30% yield, respectively.[16,18] Synthesis of monomer **13** was carried out by treatment of phloroisovalerophenone (**11**) with Vilsmeier–Haack reagent ($POCl_3$, DMF)[19] in ethyl acetate resulting in formation of 3-formyl phloroisovalerophenone (**13**)[16] in 70% yield. After synthesis of 2,4-diacetyl phloroglucinol (**7**), the key step involved in the synthesis of dimeric phloroglucinols is condensation of two monomeric units with the use of proper linker molecule. Several experimental conditions were attempted for condensation of two monomeric units as shown in Table 1. Reaction of **7** with formaldehyde in presence of *p*-TsCl in chloroform at 50 °C resulted in formation of expected product **14**[20] in a yield of 35%. The reaction was not complete in the absence of catalyst and product was obtained in a yield of 10%. Higher yields were obtained when reaction was performed under microwave irradiation (750 W, 10–15 min). Initially when reaction was performed without catalyst under MW conditions for 10 min, 35% product was obtained while reaction under neat conditions (absence of solvent as well as catalyst) at 750 W for 15 min resulted in formation of expected product in a yield of 38%. Synthesis of methylene-bis-(3,5-diacetyl-2,4,6-trihydroxy benzene) (**14**)[21] along with its isovaleryl isobutyryl and isovaleryl formyl analogues methylene-bis-(3,5-di-isopentanoyl-2,4,6-trihydroxybenzene) (**15**)[22], methylene-bis-(3,5-di-isobutanoyl-2,4,6-trihydroxybenzene) (**16**)[23,24] and methylene-bis-(3-formyl-5-isopentanoyl-2,4,6-trihydroxybenzene) (**32**)[25] from their respective monomeric precursors **7–9** and **13** is depicted in Scheme 1. Several new dimeric compounds were synthesized by varying the substitution on aromatic ring (changing acyl functionalities) and at methylene bridge (by using different aldehydes viz saturated, aromatic and heteroaromatic) in a key condensation step as shown in Scheme 1. Treatment of diacyl phloroglucinols **7** and **8** with aliphatic aldehydes viz formaldehyde, propionaldehyde, cyclohexane carbaldehyde and dihydrocinnamaldehyde under neat conditions and microwave irradiation (750 W) for 15 min resulted in formation of desired dimers **17–20** in 32–45% yield. Reaction of **7** and **8** with aromatic and heteroaromatic aldehydes under neat conditions did not yield the desired products. However, the desired dimers **21–31** were obtained in 42–50% yield in the presence of catalyst *p*-TsCl and chloroform as solvent. Dimeric phloroglucinol compounds **32–34** with formyl functionalities on aromatic ring were synthesized by reaction of **13** with formaldehyde, pyridine-2-carboxyaldehyde and 4-benzyloxybenzaldehyde and *p*-TsCl catalyst in chloroform under microwave conditions for 5–10 min. The structures of synthesized compounds are shown in Figure 3. All compounds were purified by silica gel (# 60–120) column chromatography or chromatography using RP-C_{18} silica gel and characterized by UV, IR, MS, ^{1}H NMR and ^{13}C NMR spectral data.

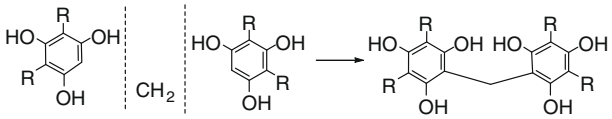


Figure 2. Proposed biogenesis of dimeric phloroglucinols.

2.2. Assay for in vitro anti-HIV activity

Synthesized dimeric phloroglucinols were initially tested for their cytotoxicity in MTT assay[26] before testing for cell based anti-HIV activity. The non-toxic concentrations of dimers were used for further screening. The anti-HIV activity assay of compounds was carried out in CEM-GFP T cells infected with HIV-1 $NL_{4.3}$ virus. CEM-GFP is a human CD4+ reporter T cell line, which expresses GFP (green fluorescent protein) upon HIV infection due to transactivation by Tat protein of stably integrated long terminal repeat regulated GFP gene.[27] This cell line is used widely for determination of anti-HIV activity due to easy visualization of infected cells.[28] The results of cytotoxicity and anti-HIV activity are shown in Table 2. Initially all the synthesized derivatives were tested for

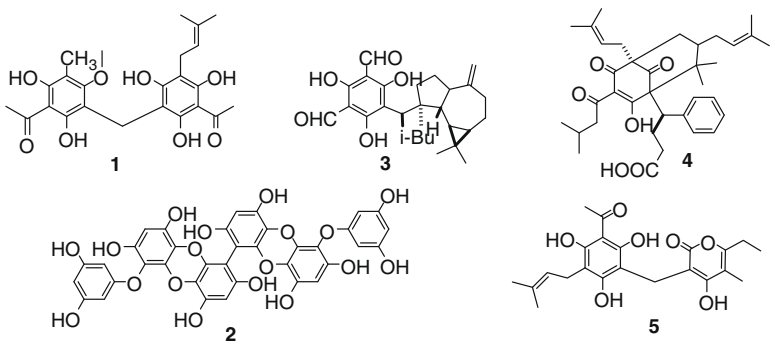
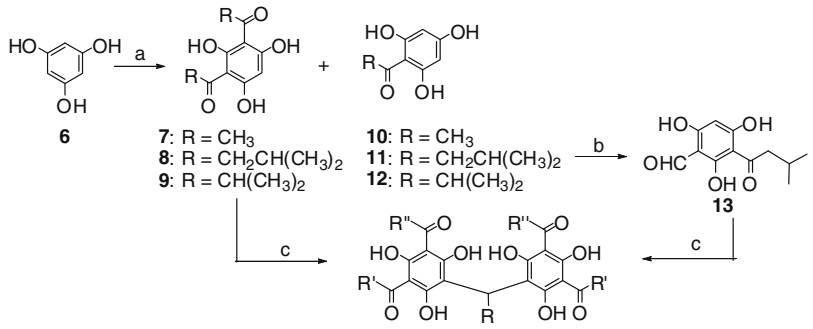


Figure 1. Anti-HIV phloroglucinol derivatives from plants.



Scheme 1. Reagents and conditions: (a) RCOCl, $AlCl_3$, $PhNO_2$, 1 h, 50 °C, 70%; (b) $POCl_3$/DMF (1 equiv each), EtOAc, rt, 2 h, 70%; (c) RCHO, MW, 750 W, 15 min, 32–45%/RCHO, *p*-TsCl, $CHCl_3$, MW, 750 W, 15 min, 42–50%.

Table 1
Condensation of two units of **7** using formaldehyde under different reaction conditions

Reagent/solvent	Reaction condition	Yield[a] (%)
$CHCl_3$, *p*-TsCl	rt, 2 h	0
$CHCl_3$, *p*-TsCl	50 °C, 2 h	35
THF, *p*-TsCl	50 °C, 2 h	15
$PhCH_3$, *p*-TsCl	60 °C, 2 h	15
CH_3OH, KOH	60 °C, 2 h	0
$CHCl_3$	50 °C, 2 h	10
$CHCl_3$, *p*-TsCl	MW, 750 W, 10 min	25
$CHCl_3$	MW, 750 W, 10 min	35
Neat	MW, 750 W, 15 min	38

[a] The yields are reported less than 50% as two equivalents of monomeric starting material produce 1 equiv of dimeric product.

anti-HIV activity at highest non-toxic concentration. Seven out of the 21 compounds showed 60% or more inhibition of HIV replication in CEM-GFP cells. These seven compounds were analyzed further for determination of CC_{50} and IC_{50} in order to look for a potential lead molecule for further development. In order to find mechanism of action, these seven active compounds were further tested for reverse transcriptase inhibitory activity.

2.3. Structure–activity relationship (SAR)

We synthesized molecules with different aromatic and heteroaromatic substituents on linker methylene carbon and also changed two of the acyl groups on aromatic phloroglucinol moieties with two formyl groups to study the changes in anti-HIV activity. First, four different variations on phloroglucinol nucleus were tried. Diisovaleryl, diisobutyryl, diacetyl and formyl-isovaleryl groups were introduced onto the aromatic nuclei. All the four compounds (**14**–**16** and **32**) were inactive in the anti-HIV assay. Next, different alkyl, aromatic, heteroaromatic were introduced on the linker methylene bridge. Results indicate that although analogue

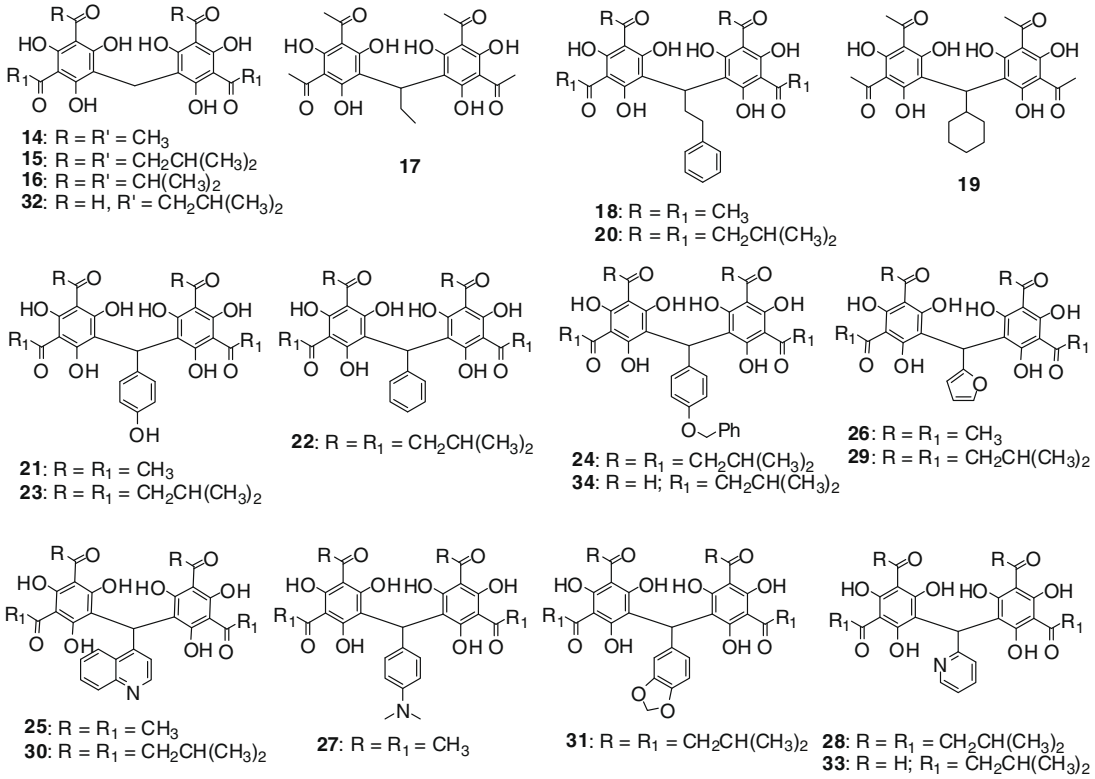


Figure 3. Synthesized dimeric phloroglucinol compounds.

Table 2
Anti-HIV activity of phloroglucinol derivatives as analyzed by p24 antigen production in HIV-1 $NL_{4.3}$ infected CEM-GFP T cells due to treatment with the compounds

Code	Highest non-toxic concentration (μM)	% Inhibition (p24 assay)	IC_{50}[a] (μM)	CC_{50}[b] (μM)
14	0.23	0	ND	ND
15	0.17	0	ND	ND
16	0.09	0	ND	ND
17	2.17	17.5 ± 0.09	ND	ND
18	1.87	16 ± 0.1	ND	ND
19	9.73	78.5 ± 5.7	4.09 ± 0.58	58.93 ± 6.34
20	0.71	15 ± 0.08	ND	ND
21	14.31	56 ± 6.8	ND	ND
22	0.74	66.5 ± 8.2	0.28 ± 0.07	3.15 ± 0.77
23	1.45	15.6 ± 0.3	ND	ND
24	5.75	70.0 ± 3.9	2.71 ± 0.49	24.00 ± 4.13
25	35.78	71.1 ± 4.6	18.59 ± 1.97	93.35 ± 4.1
26	15.06	73 ± 11.4	6.75 ± 0.33	61.18 ± 6.12
27	9.07	60.6 ± 7.5	6.06 ± 0.63	22.07 ± 3.9
28	7.39	64.7 ± 12.8	3.37 ± 0.79	38.05 ± 3.64
29	3.75	56.8 ± 7.3	ND	ND
30	3.44	30.1 ± 11.6	ND	ND
31	2.77	13.9 ± 0.05	ND	ND
32	4.10	0	ND	ND
33	15.91	26.9 ± 0.02	ND	ND
34	7.46	0	ND	ND
AZT	5.00	89.75 ± 7.07	1.05 ± 0.07	24.06 ± 0.63

[a] IC_{50} = concentration of compound to achieve 50% inhibition of infected cells (n = 3).
[b] CC_{50} = concentration of compound indicating cytotoxicity against noninfected cells (n = 3); ND = not determined.

25 was least toxic with CC_{50} of 93.35 μM, it exhibited weaker activity. Among the seven active compunds, **22** with phenyl group on linker methylene and two diisovaleryl phloroglucinol moieties was found to be most active having an IC_{50} of 0.28 μM. This compound showed a CC_{50} of 3.15 μM indicating a good safety index for further analysis as a potential lead molecule. Activity and cytotoxicity was diminished when phenyl moiety of **22** was substituted with hydroxyl group at para position as observed in **23**. Good activity was observed in **25**–**28** where phenyl moiety was replaced with heteroaromatic moiety like quinoline-4-yl, furan-2-yl, 4-N, *N*-dimethyl phenyl-1-yl, and pyridine-2-yl. Though these compounds showed weaker activity compared to **22**, but they have better safety index. Compounds **14**–**16** and **32** which are devoid of substitution on linker methylene carbon were inactive and highly toxic to the cells. Replacing acyl functionality in **24** and **28** with formyl group also resulted in loss of activity as observed in **34** and **33**, respectively. Dimeric phloroglucinols with directly attached aromatic ring or bulky alkyl substitution on linker methylene carbon exhibited better anti-HIV activity as compared to simple aliphatic substituents.

SAR study established the basic pharmacophore responsible for activity (Fig. 4). Dimeric phloroglucinols with four acyl substituents and linker methylene carbon substituted with bulky aromatic group were found to be essential for anti-HIV activity.

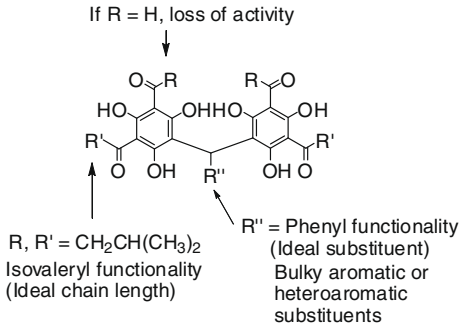


Figure 4. Structure–activity relationship of dimeric phloroglucinols.

2.4. In vitro HIV-1 reverse transcriptase assay

Active analogues can inhibit the virus by targeting different steps in virus life cycle or enzymes like, entry, reverse transcriptase, integrase, protease and viral assembly. Seven dimeric phloroglucinols showing activity in cell based assay were further tested for reverse transcriptase inhibition as similar compounds were earlier shown to be RT inhibitors. Active analogues **19**, **22** and **24**–**28** were evaluated for in vitro reverse transcriptase activity (Table 3) and Nevirapine a known reverse transcriptase inhibitor was used as positive standard. Analogues **24** and **25** showed 87.75% and 80.46% inhibition of reverse transcriptase enzyme, respectively, while **26** and **27** showed weak inhibition of reverse transcriptase suggesting multiple modes of inhibition. The most active compound **22** showed very weak activity at 20 μg/mL concentration indicating an alternative mechanism of viral inhibition.

3. Conclusion

Twenty-one dimeric phloroglucinols have been biomimetically synthesized and screened for in vitro anti-HIV assay. Structure–activity relationships for HIV-1 inhibition of this class of compounds were elucidated. Compounds **19**, **22** and **24**–**28** exhibited moderate to good anti-HIV activity in HIV-1 $NL_{4.3}$ infected CEM-GFP T cells (Table 2). Seven dimeric phloroglucinols showing activities in initial assays were further screened for inhibition of reverse transcriptase enzyme. Compound **22** which is diisovaleryl phloroglucinol dimer with phenyl substituent at methylene bridge was found to be the most active in HIV-1 $NL_{4.3}$ infected CEM-GFP T cells having an IC_{50} of 0.28 μM, thus showing better inhibitory activity than AZT in the cell based assay. This compound showed a CC_{50} of 3.15 μM indicating a good safety index for further analysis as a potential lead molecule. Analogues **24** and **25** showed 87.75 and 80.46% inhibition of reverse transcriptase, respectively, while the most active compound **22** showed very weak inhibition of RTase suggesting an alternate mechanism of inhibition. It is also possible that these compounds get modified inside the cell to become potent RT inhibitor as is the case with AZT, which needs to be phosphorylated to become active.[29] It is suggested that few of these dimeric phloroglucinol compounds, especially **22** could be used as a lead molecule for further development of new anti-HIV therapeutic molecule.

4. Experimental

Melting points were recorded on capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on 300 MHz Bruker FT-NMR (Avance DPX300) spectrometer using tet-

Table 3
Reverse transcriptase inhibitor activity due to treatment with the compounds

Code	Concentration (μg/reactions)	% Reverse transcriptase inhibition	EC_{50}[a] (μM)
19	20	0	ND
22	20	4.61 ± 0.7	ND
24	20	87.75 ± 4.65	1.035 ± 0.012
25	20	80.46 ± 1.06	0.47 ± 0.0.04
26	20	16.22 ± 0.1	ND
27	20	13.39 ± 0.06	ND
28	20	0	ND
Nevirapine	0.1	95.5 ± 0.1	0.0036 ± 0.0001

[a] EC_{50} = concentration of compound to achieve 50% inhibition of reverse transcriptase (n = 3); ND = not determined.

ramethylsilane as internal standard and the chemical shifts are reported in δ units. Mass spectra were recorded on either GC–MS (Shimadzu QP 5000 spectrometer) auto sampler/direct injection (EI/CI) or LC–MS (APCI/ ESI). Domestic microwave oven (Whirlpool, Sweden, Model: MT-243) was used to carry out microwave heated reactions. All chromatographic purifications were performed with silica gel (60–120 mesh), whereas all TLC (silica gel) development was performed on silica gel coated (Merck Kiesel 60F_{254}, 0.2 mm thickness) sheets. All chemicals were purchased from Sigma-Aldrich, SD fine chemicals, Lancaster and CDH. Solvents used for the chemical synthesis purchased from commercial sources were of analytical grade and were used without further purification unless otherwise stated.

4.1. General method for synthesis of dimeric phloroglucinols 14–20

The mixture of diacyl phloroglucinol (**7** or **8** 1 mmol) and aldehyde (0.5 mmol) was irradiated with microwave radiations (750 W) in domestic microwave oven for 10–15 min. On cooling, the reaction mixture was diluted with chloroform. The resultant mixture was washed with water and brine solution and finally dried over anhydrous sodium sulfate. Solvent was removed under vacuum and the crude product was purified by silica gel (# 60–120) column chromatography using hexane–EtOAc as eluent.

4.1.1. Methylene-bis-(3,5-diacetyl-2,4,6-trihydroxy benzene) (14)

Yield: 35%; cream colored solid; mp 278–280 °C; UV ($CHCl_3$): λ_{max} (log ε) 275 (4.34), 336 (3.88); IR (KBr): ν_{max} 3201, 1616, 1594, 1477, 1424, 1370, 1268, 1181, 1132, 1112, 1022 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 17.38 (s, 2H, 2 × O*H*), 16.22 (s, 2H, 2 × O*H*), 10.23 (s, 2H, 2 × O*H*), 3.74 (s, 2H), 2.76 (s, 6H), 2.73 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 205.8, 171.5, 169.1, 166.5, 106.6, 105.8, 104.9, 34.1, 33.0, 15.6; EIMS: *m/z* 432 $[M]^+$, 223, 210, 195, 177, 85, 69, 67.

4.1.2. Methylene-bis-(3,5-di-isopentanoyl-2,4,6-trihydroxybenzene) (15)

Yield: 38%; yellow solid; mp 158–160 °C; UV ($CHCl_3$): λ_{max} (log ε) 282 (4.44), 339 (3.97); IR (KBr): ν_{max} 3201, 2959, 2872, 1622, 1545, 1470, 1427, 1367, 1302, 1204, 1170, 1131, 1075 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 17.64 (s, 2H, 2 × O*H*), 16.45 (s, 2H, 2 × O*H*), 10.32 (s, 2H, 2 × O*H*), 3.71 (s, 2H), 3.04 (d, *J* = 6.4 Hz, 8H), 2.27 (m, 4H), 1.00 (d, *J* = 6.4 Hz, 24H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 208.3, 207.8, 171.3, 168.5, 165.5, 105.3, 105.1, 104.7, 53.7, 52.9, 25.6, 23.3, 16.0; CIMS: *m/z* 601 $[M+1]^+$, 307 ($M-C_{16}H_{22}O_5$), 295. Anal. Calcd for $C_{33}H_{44}O_{10}$ (600.3): C, 65.98; H, 7.38. Found: C, 65.81; H, 7.45.

4.1.3. Methylene-bis-(3,5-di-isobutanoyl-2,4,6-trihydroxybenzene) (16)

Yield: 34%; light orange solid; mp 200–202 °C; UV ($CHCl_3$): λ_{max} (log ε) 272 (4.33), 341 (3.73); IR (KBr): ν_{max} 3188, 2970, 1618, 1467, 1382, 1273, 1211, 1160, 1065; ^1H NMR ($CDCl_3$, 300 MHz): δ 17.73 (s, 2H, 2 × O*H*), 16.51 (s, 2H, 2 × O*H*), 10.39 (s, 2H, 2 × O*H*), 4.04 (m, 4H), 3.76 (s, 2H), 1.22 (d, *J* = 5.9 Hz, 24H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 213.1, 212.8, 171.3, 168.8, 165.2, 105.3, 104.4, 103.8, 40.1, 39.7, 20.0, 19.6, 16.2; CIMS: *m/z* 545 $[M+1]^+$, 279, 267. Anal. Calcd for $C_{29}H_{36}O_{10}$ (544.2): C, 63.96; H, 6.66. Found: C, 64.06; H, 6.78.

4.1.4. 1-[3-Acetyl-5-[(3,5-diacetyl-2,4,6-trihydroxyphenyl) ethylmethyl]-2,4,6-trihydroxyphenyl]ethanone (17)

Yield: 45%; yellow solid; mp 178–180 °C; UV ($CHCl_3$): λ_{max} (log ε) 272 (4.27), 336 (3.50); IR (KBr): ν_{max} 3437, 2928, 1697, 1618, 1581, 1462, 1404, 1259, 1046 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 17.73 (s, 1H, O*H*), 17.49 (s, 1H, O*H*), 16.25 (s, 2H, 2 × O*H*), 10.78 (s, 1H, O*H*), 9.95 (s, 1H, O*H*), 4.42 (t, *J* = 7.2 Hz, 1H), 2.76 (s, 6H), 2.73 (s, 6H), 2.31 (m, 2H), 0.88 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 205.7, 171.2, 166.4, 164.5, 107.5, 106.4, 104.2, 34.2, 33.0, 32.4, 24.3, 14.1; CIMS: *m/z* 461 $[M+1]^+$, 251 $[M-C_{10}H_{10}O_5]$. Anal. Calcd for $C_{23}H_{24}O_{10}$ (460.1): C, 60.00; H, 5.25. Found: C, 59.89; H, 5.19.

4.1.5. 1-[3-Acetyl-5-[(3,5-diacetyl-2,4,6-trihydroxyphenyl)-2-phenylethyl-methyl]-2,4,6-trihydroxyphenyl]ethanone (18)

Yield: 33%; cream colored solid; mp 188–190 °C; UV ($CHCl_3$): λ_{max} (log ε) 272 (4.42), 341 (3.78); IR (KBr): ν_{max} 3204, 1615, 1578, 1473, 1417, 1363, 1320, 1264 1109 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 17.86 (s, 1H, O*H*), 17.46 (s, 1H, O*H*), 16.25 (s, 2H, 2 × O*H*), 10.86 (s, 1H, O*H*), 9.90 (s, 1H, O*H*), 7.26–7.12 (m, 3H), 7.08 (d, *J* = 6.8 Hz, 2H), 4.53 (t, *J* = 7.1 Hz, 1H), 2.76 (s, 6H), 2.72 (s, 6H), 2.56 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 205.7, 171.3, 169.4, 168.5, 166.4, 141.5, 129.0, 128.9, 126.6, 105.0, 104.7, 104.5, 35.7, 34.1, 32.9, 32.7, 29.9; CIMS: *m/z* 537 $[M+1]^+$, 327 $[M-C_{10}H_{10}O_5]$. Anal. Calcd for $C_{29}H_{28}O_{10}$ (536.2): C, 64.92; H, 5.26. Found: C, 65.01; H, 5.18.

4.1.6. 1-[3-Acetyl-5-[(3,5-diacetyl-2,4,6-trihydroxyphenyl)-cyclohexyl-methyl]-2,4,6-trihydroxyphenyl]ethanone (19)

Yield: 35%; cream colored solid; mp 196–198 °C; UV ($CHCl_3$): λ_{max} (log ε) 276 (4.29), 341 (3.59); IR (KBr): ν_{max} 3173, 2915, 2849, 1617, 1583, 1473, 1406, 1366, 1259, 1167, 1108 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 17.78 (s, 1H, O*H*), 17.46, s, 1H, O*H*), 16.25 (s, 2H, 2 × O*H*), 10.86 (s, 1H, O*H*), 9.96 (s, 1H, O*H*), 4.17 (d, *J* = 10.9 Hz, 1H), 2.76 (s, 6H), 2.72 (s, 6H), 1.64 (m, 1H), 1.26–1.10 (m, 6H), 0.92–0.70 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 205.6, 205.2, 169.7, 167.4, 167.1, 165.7, 165.2, 106.4, 106.0, 105.2, 103.9, 36.7, 36.2, 33.8, 32.8, 32.4, 26.3, 26.0; CIMS: *m/z* 515 $[M+1]^+$, 305 $[M-C_{10}H_{10}O_5]$. Anal. Calcd for $C_{27}H_{30}O_{10}$ (514.2): C, 63.03; H, 5.88. Found: C, 62.98; H, 5.72.

4.1.7. 1-[3-Isopentanoyl-5-[(3,5-diisopentanoyl-2,4,6-trihydroxyphenyl)-phenyl-ethyl-methyl]-2,4,6-trihydroxyphenyl]-3-methylbutan-1-one (20)

Yield: 32%; yellow solid; mp 138–140 °C; UV ($CHCl_3$): λ_{max} (log ε) 285 (4.55), 341 (4.05); IR (KBr): ν_{max} 3202, 2958, 1615, 1580, 1469, 1392, 1367, 1299, 1201, 1128, 1054 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 18.13 (s, 1H, O*H*), 17.76 (s, 1H, O*H*), 16.46 (s, 2H, 2 × O*H*), 10.96 (s, 1H, O*H*), 10.03 (s, 1H, O*H*), 7.26–7.12 (m, 3H), 7.08 (d, *J* = 7.6 Hz, 2H), 4.54 (t, *J* = 7.1 Hz, 1H), 3.05 (d, *J* = 6.5 Hz, 8H), 2.31 (m, 4H), 2.27 (m, 4H), 1.01 (d, *J* = 6.4 Hz, 24H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 208.7, 208.0, 171.2, 169.7, 168.8, 166.2, 166.0, 141.7, 128.9, 126.5, 108.0, 105.7, 104.7, 53.9, 52.9, 35.8, 32.8, 30.3, 25.7, 23.3; CIMS: *m/z* 411 $[M-C_{10}H_{10}O_5]$. Anal. Calcd for $C_{41}H_{52}O_{10}$ (704.4): C, 69.86; H, 7.44. Found: C, 69.75; H, 7.53.

4.2. General method for synthesis of dimeric phloroglucinols 21–31

To the solution of diacyl phloroglucinols (**7, 8** 1 mmol) in chloroform, *p*-TsCl and aldehyde (0.5 mmol) was added and the reaction mixture was irradiated with microwave radiations (750 W) in domestic microwave oven for 10–15 min. Remaining procedure is same as given for **14–20**.

4.2.1. 1-[3-Acetyl-5-[(3,5-diacetyl-2,4,6-trihydroxyphenyl)-4-hydroxyphenyl-methyl]-2,4,6-trihydroxyphenyl]ethanone (21)

Yield: 50%; light brown solid; mp 110–112 °C; UV ($CHCl_3$): λ_{max} (log ε) 272 (4.20), 339 (3.31); IR (KBr): ν_{max} 3249, 2928, 1615,

1511, 1425, 1366, 1266, 1111 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 17.63 (s, 2H, 2 × O*H*), 16.36 (s, 2H, 2 × O*H*), 10.18 (s, 2H, 2 × O*H*), 6.95 (d, *J* = 7.8 Hz, 2H), 6.76 (d, *J* = 7.9 Hz, 2H), 6.09 (s, 1H), 5.11 (br s, 1H), 2.78 (s, 6H), 2.72 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 205.5, 205.3, 171.0, 168.6, 166.0, 154.2, 132.4, 127.8, 115.3, 106.1, 105.3, 104.3, 33.5, 32.4, 29.7; CIMS: *m/z* 315 [M−$C_{10}H_{10}O_5$]. Anal. Calcd for $C_{27}H_{24}O_{11}$ (524.1): C, 61.83; H, 4.61. Found: C, 61.91; H, 4.56.

4.2.2. 1-[3-Isopentanoyl-5-[(3,5-diisopentanoyl-2,4,6-trihydroxyphenyl)-phenyl-methyl]-2,4,6-trihydroxyphenyl]-3-methylbutan-1-one (22)

Yield: 43% yellow solid; mp 180–182 °C; UV ($CHCl_3$): λ_{max} (log ε) 272 (4.52), 340 (3.70); IR (KBr): ν_{max} 2957, 1685, 1617, 1583, 1454, 1424, 1326, 1293, 1186, 1128, 1070, 1027 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 17.90 (s, 2H, 2 × O*H*), 16.55 (s, 2H, 2 × O*H*), 10.26 (s, 2H, 2 × O*H*), 7.30–7.23 (m, 3H), 7.10 (d, *J* = 7.5 Hz, 2H), 6.16 (s, 1H), 3.06 (d, *J* = 16.3 Hz, 8H), 2.26 (m, 4H), 1.00 (d, *J* = 6.4 Hz, 24H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 208.3, 171.5, 169.5, 166.2, 137.2, 128.9, 127.1, 126.9, 107.0, 105.8, 104.9, 53.9, 52.9, 30.2, 25.7, 23.3; CIMS: *m/z* 384 [M−$C_{10}H_{10}O_5$]. Anal. Calcd for $C_{39}H_{48}O_{10}$ (676.3): C, 69.21; H, 7.15. Found: C, 69.10; H, 7.28.

4.2.3. 1-[3-Isopentanoyl-5-[(3,5-diisopentanoyl-2,4,6-trihydroxyphenyl)-4-hydroxyphenyl-methyl]-2,4,6-trihydroxyphenyl]-3-methylbutan-1-one (23)

Yield: 50%; brown oil; UV ($CHCl_3$): λ_{max} (log ε) 280 (4.51), 340 (3.72); IR (Neat): ν_{max} 3433, 2978, 1756, 1698, 1615, 1581, 1510, 1456, 1298, 1196, 1123, 1092 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 17.87 (br s, 2H, 2 × O*H*), 16.53 (s, 2H, 2 × O*H*), 10.24 (br s, 2H, 2 × O*H*), 6.96 (d, *J* = 8.1 Hz, 2H), 6.77 (d, *J* = 8.4 Hz, 2H), 6.08 (s, 1H), 3.05 (d, *J* = 10.6 Hz, 8H), 2.27 (m, 4H), 0.99 (d, *J* = 6.7 Hz, 24 H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 208.4, 171.5, 169.5, 166.2, 154.8, 128.8, 128.4, 114.6, 106.9, 105.8, 104.9, 52.6, 52.9, 25.8, 23.3, 14.7; CIMS: *m/z* 400 [M−$C_{10}H_{10}O_5$]. Anal. Calcd for $C_{39}H_{48}O_{11}$ (692.3): C, 67.61; H, 6.98. Found: C, 67.49; H, 7.07.

4.2.4. 1-[3-Isopentanoyl-5-[(3,5-diisopentanoyl-2,4,6-trihydroxyphenyl)-4-benzyloxyphenyl-methyl]-2,4,6-trihydroxyphenyl]-3-methylbutan-1-one (24)

Yield: 42%; brown crystals; mp 152–154 °C; UV ($CHCl_3$): λ_{max} (log ε) 272 (3.24), 340 (3.72); IR (KBr): ν_{max} 3253, 2965, 1750, 1652, 1620, 1171, 1120, 1095 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 17.87 (br s, 2H, 2 × OH), 16.53 (s, 2H, 2 × OH), 10.23 (br s, 2H, 2 × OH), 7.43–7.35 (m, 5H), 6.99 (d, *J* = 8.7 Hz, 2H), 6.88 (d, *J* = 8.7 Hz, 2H), 6.09 (s, 1H), 5.04 (s, 1H), 3.05 (d, *J* = 16.5 Hz, 8H), 2.27 (m, 4H), 0.98 (d, *J* = 6.7 Hz, 24 H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 208.3, 208.1, 171.5, 169.4, 166.1, 157.9, 137.5, 129.5, 129.1, 128.5, 128.2, 128.0, 115.1, 106.8, 105.7, 104.8, 70.5, 53.8, 52.9, 33.2, 25.7, 23.3; EIMS: *m/z* 489.13 [M-$C_{16}H_{22}O_5$]; 295.13. Anal. Calcd for $C_{46}H_{54}O_{11}$ (782.9): C, 70.57; H, 6.95. Found: C, 70.52; H, 7.26.

4.2.5. 1-[3-Acetyl-5-[(3,5-diacetyl-2,4,6-trihydroxyphenyl)-quinolin-4-yl-methyl]-2,4,6-trihydroxyphenyl]ethanone (25)

Yield: 48%; white solid; mp 196–198 °C; UV ($CHCl_3$): λ_{max} (log ε) 288 (4.44); IR (KBr): ν_{max} 2930, 1621, 1596, 1509, 1424, 1364, 1271, 1183, 1107 cm^{-1}; ^1H NMR (pyridine-d_5, 300 MHz): δ 8.94 (d, J = 3.9 Hz, 1H), 8.24 (m, 4H), 7.08 (d, J = 8.4 Hz, 1H), 4.62 (s, 1H), 2.79 (s, 6H), 2.71 (s, 6H); ^{13}C NMR (pyridine-d_5, 75 MHz): δ 205.1, 170.5, 131.3, 130.8, 128.4, 126.4, 118.4, 106.8, 34.0, 33.5, 33.3; EIMS: 559 [M]+, 348, 209. Anal. Calcd for $C_{30}H_{25}NO_{10}$ (559.2): C, 64.40; H, 4.50; N, 2.50. Found: C, 64.51; H, 4.63; N, 2.39.

4.2.6. 1-[3-Acetyl-5-[(3,5-diacetyl-2,4,6-trihydroxyphenyl)-furan-2-yl-methyl]-2,4,6-trihydroxyphenyl]ethanone (26)

Yield: 42%; light green solid; mp 143–145 °C; UV ($CHCl_3$): λ_{max} (log ε) 279 (4.34), 336 (3.80); IR (KBr): ν_{max} 3216, 1618, 1580, 1412, 1364, 1261, 1185, 1114, 1020 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 17.61 (s, 2H, 2 × O*H*), 16.34 (s, 2H, 2 × O*H*), 10.13 (s, 2H, 2 × O*H*), 7.31 (s, 1H), 6.32 (s, 1H), 6.00 (s, 2H), 2.76 (s, 6H), 2.73 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 206.0, 205.6, 171.7, 168.9, 166.1, 151.0, 142.3, 110.8, 107.3, 105.7, 105.3, 104.8, 34.0, 33.9, 29.6; CIMS: *m/z* 499 $[M+1]^+$, 289 [M-$C_{10}H_{10}O_5$]. Anal. Calcd for $C_{25}H_{22}O_{11}$ (498.1): C, 60.24; H, 4.45. Found: C, 60.12; H, 4.57.

4.2.7. 1-[3-Acetyl-5-[(3,5-diacetyl-2,4,6-trihydroxyphenyl)-4-*N*,*N*-dimethyl-aminophenyl-methyl]-2,4,6-trihydroxyphenyl]ethanone (27)

Yield: 48%; white solid; mp 196–198 °C; UV ($CHCl_3$): λ_{max} (log ε) 288 (4.44); IR (KBr): ν_{max} 2930, 1621, 1596, 1509, 1424, 1364, 1271, 1183, 1107 cm^{-1}; ^1H NMR (pyridine-d_5, 300 MHz): δ 8.94 (d, *J* = 3.9 Hz, 1H), 8.24 (m, 4H), 7.08 (d, *J* = 8.4 Hz, 1H), 4.62 (s, 1H), 2.79 (s, 6H), 2.71 (s, 6H); ^{13}C NMR (pyridine-d_5, 75 MHz): δ 205.1, 170.5, 131.3, 130.8, 128.4, 126.4, 118.4, 106.8, 34.0, 33.5, 33.3; EIMS: 559 $[M]^+$, 348, 209. Anal. Calcd for $C_{30}H_{25}NO_{10}$ (559.2): C, 64.40; H, 4.50; N, 2.50. Found: C, 64.51; H, 4.63; N, 2.39.

4.2.8. 1-[3-Isopentanoyl-5-[(3,5-diisopentanoyl-2,4,6-trihydroxyphenyl)-pyridin-2-yl-methyl]-2,4,6-trihydroxyphenyl]-3-methylbutan-1-one (28)

Yield: 46%; yellow solid; mp 190–192 °C; UV ($CHCl_3$): λ_{max} (log ε) 282 (4.53), 340 (3.95); IR (KBr): ν_{max} 3436, 2957, 2870, 1618, 1545, 1458, 1366, 1302, 1202, 1130, 1063 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 16.63 (s, 2H, 2 × O*H*), 8.42 (d, *J* = 5.1 Hz, 1H), 8.09 (t, *J* = 7.4 Hz, 1H), 7.89 (d, *J* = 8.1 Hz, 1H), 7.57 (t, *J* = 9.2 Hz, 1H), 7.07 (s, 1H), 3.15–2.84 (m, 8H), 2.21 (m, 4H), 0.97 (d, *J* = 6.6 Hz, 24H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 208.0, 206.9, 173.1, 170.8, 162.4, 144.2, 140.7, 127.4, 123.7, 106.6, 105.7, 104.8, 53.3, 34.3, 34.3, 26.0, 23.5, 23.3; CIMS: *m/z* 384 [M−$C_{10}H_{10}O_5$]. Anal. Calcd for $C_{38}H_{47}NO_{10}$ (677.3): C, 67.34; H, 6.99; N, 2.07. Found: C, 67.47; H, 6.89; N, 1.97.

4.2.9. 1-[3-Isopentanoyl-5-[(3,5-diisopentanoyl-2,4,6-trihydroxyphenyl)-furan-2-yl-methyl]-2,4,6-trihydroxyphenyl]-3-methylbutan-1-one (29)

Yield: 48%; yellow oil; UV ($CHCl_3$): λ_{max} (log ε) 288 (4.54), 340 (3.98); IR (Neat): ν_{max} 3437, 2958, 1618, 1466, 1299, 1118, 1046 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 17.88 (s, 2H, 2 × O*H*), 16.56 (s, 2H, 2 × O*H*), 10.19 (s, 2H, 2 × O*H*), 7.32 (s, 1H), 6.33 (d, *J* = 2.0 Hz, 1H), 6.01 (s, 1H), 5.80 (s, 1H), 3.03 (m, 8H), 2.26 (m, 4H), 1.00 (d, *J* = 3.2 Hz, 24H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 208.4, 208.1, 171.7, 169.2, 165.8, 151.3, 142.2, 110.8, 107.3, 105.2, 104.8, 95.9, 53.8, 53.3, 52.9, 30.2, 29.9, 25.7, 23.3; CIMS: *m/z* 374 [M−$C_{10}H_{10}O_5$]. Anal. Calcd for $C_{37}H_{46}O_{11}$ (666.3): C, 66.65; H, 6.95. Found: C, 66.80; H, 7.08.

4.2.10. 1-[3-Isopentanoyl-5-[(3,5-diisopentanoyl-2,4,6-trihydroxyphenyl)-quinolin-4-yl-methyl]-2,4,6-trihydroxyphenyl]-3-methylbutan-1-one (30)

Yield: 48%; cream colored solid; mp 200–202 °C; UV ($CHCl_3$): λ_{max} (log ε) 276 (4.65), 340 (4.02); IR (KBr): ν_{max} 2957, 2870, 2545, 1615, 1418, 1367, 1301, 1198, 1127 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 16.49 (br s, 3H, 3 × O*H*), 16.16 (s, 2H, 2 × O*H*), 13.84 (br s, 1H, O*H*), 8.25 (s, 2H), 7.76–7.48 (m, 4H), 7.26 (s, 1H), 3.04 (br s, 8H), 2.29 (m, 4H), 1.01 (d, *J* = 4.4 Hz, 24H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 207.1, 172.2, 170.4, 170.1, 140.1, 137.9, 127.7, 120.7, 10.5.1, 104.4, 103.9, 53.4, 34.1, 25.6, 23.4; CIMS: *m/z* 727 $[M+1]^+$. Anal. Calcd for $C_{42}H_{49}NO_{10}$ (727.3): C, 69.31; H, 6.79; N, 1.92. Found: C, 69.19; H, 6.65; N, 1.79.

4.2.11. 1-[3-Isopentanoyl-5-[(3,5-diisopentanoyl-2,4,6-trihydroxyphenyl)-3,4-methylenedioxyphenyl -methyl]-2,4,6-trihydroxyphenyl]-3-methylbutan-1-one (31)

Yield: 45%; yellow crystalline solid; mp 172–174 °C; UV ($CHCl_3$): $\lambda_{max\ (\log \varepsilon)}$ 267 (2.94); IR (KBr): ν_{max} 3201, 2958, 1614, 1583, 1504, 1487, 1367, 1200, 1127, 1041 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 17.9 (br s, 2H, 2 × O*H*), 16.56 (s, 2H, 2 × O*H*), 10.24 (br s, 2H, 2 × O*H*), 6.71 (d, *J* = 8.1 Hz, 1H), 6.58 (s, 1H), 6.55 (d, *J* = 8.7 Hz, 1H), 6.07 (s, 1H), 5.94 (s, 1H), 3.04 (d, *J* = 14.4 Hz, 8H), 2.26 (m, 4H), 0.99 (d, *J* = 6.7 Hz, 24 H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 208.4, 208.1, 171.5, 169.3, 166.1, 148.5, 146.6, 131.4, 119.9, 108.4, 107.9, 106.7, 105.7, 104.8, 101.6, 53.8, 52.8, 33.6, 25.7, 23.3 Maldi Tof: *m/z* 720.47. Anal. Calcd for $C_{40}H_{48}O_{12}$ (720.31): C, 66.65; H, 6.71; O, 26.64. Found: C, 66.52; H, 6.63.

4.3. General method for synthesis of dimeric phloroglucinols 32–34

To the solution of 3-formyl phloroisovalerophenone (**13**, 1 mmol) in chloroform, *p*-TsCl and aldehyde (0.5 mmol) was added and the reaction mixture was irradiated with microwave radiations (750 W) in domestic microwave oven for 10–15 min. On cooling, the reaction mixture was diluted with chloroform. The resultant mixture was washed with water and brine solution and finally dried over anhydrous sodium sulfate. Solvent was removed under vacuum and the crude product was purified by reverse phase silica gel column chromatography using MeOH and water as eluent.

4.3.1. Methylene-bis-(3-formyl-5-isopentanoyl-2,4,6-trihydroxybenzene) (32)

Yield: 30%; cream colored solid; mp 228–230 °C; UV ($CHCl_3$): λ_{max} (log ε) 284 (4.33); IR (KBr): ν_{max} 3373, 2921, 1621, 1474, 1399, 1184, 1048 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 17.65 (s, 2H, 2 × O*H*), 14.51 (s, 2H, 2 × O*H*), 10.15 (s, 2H, 2 × O*H*), 9.78 (s, 2H, 2 × C*H*O), 3.72 (s, 2H), 3.03 (d, *J* = 6.6 Hz, 4H), 2.26 (m, 2H), 1.01 (d, *J* = 6.6 Hz, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 207.6, 193.6, 171.2, 169.4, 165.5, 105.4, 105.1, 103.5, 52.6, 25.7, 23.3, 15.3; CIMS: *m/z* 489 $[M+1]^+$. Anal. Calcd for $C_{25}H_{28}O_{10}$ (488.2): C, 61.47; H, 5.78. Found: C, 61.61; H, 5.89.

4.3.2. 1-[3-Formyl-5-[(5-formyl-3-isopentanoyl-2,4,6-trihydroxyphenyl)-pyridin-2-yl-methyl]-2,4,6-trihydroxyphenyl]-3-methylbutan-1-one (33)

Yield: 44%; yellow solid; mp 148–149 °C; UV ($CHCl_3$): λ_{max} (log ε) 268 (3.40); IR (KBr): $\nu_{max;}$ 3470, 2930, 1630, 1545, 1470, 1366, 1302, 1045 cm^{-1}; ^1H NMR (CD_3OD + $CDCl_3$, 300 MHz): 9.87 (s, 2H), 8.25 (d, *J* = 5.3 Hz, 1H), 8.20 (t, *J* = 7.2 Hz, 1H), 7.64 (d, *J* = 7.5 Hz, 1H), 7.59 (t, *J* = 9.0 Hz, 1H), 7.32 (s, 1H), 2.88 (d, *J* = 5.4 Hz, 4H), 2.10 (m, 2H), 0.83 (d, *J* = 6.6 Hz, 12H); ^{13}C NMR (CD_3OD + $CDCl_3$, 75 MHz): δ 205.5, 193.4, 190.6, 171.7, 169.8, 160.2, 145.5, 140.0, 126.2, 123.6, 106.2, 104.3, 101.9, 52.32, 29.46, 25.19, 22.37; CIMS: *m/z* 328.1 $[M-C_{12}H_{13}O_5]$. Anal. Calcd for $C_{30}H_{31}NO_{10}$ (565.57): C, 67.71; H, 5.52; N, 2.48. Found: C, 67.47; H, 5.57; N, 2.43.

4.3.3. 1-[3-Formyl-5-[(5-formyl-3-isopentanoyl-2,4,6-trihydroxyphenyl)-4-benzyloxyphenyl-methyl]-2,4,6-trihydroxyphenyl]-3-methylbutan-1-one (34)

Yield: 40% cream colour soild; mp 180 °C; UV ($CHCl_3$): λ_{max} (log ε) 266 (3.35); IR (KBr): 3280, 2970, 1753, 1652, 1628, 1167, 1125, 1085 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 17.8 (br s, 2H, 2 × O*H*), 14.6 (s, 2H, 2 × O*H*), 10.16 (s, 2H), 9.63 (br s, 2H, 2 × O*H*), 7.40 (t, *J* = 9.2 Hz, 1H), 8.42 (d, *J* = 5.1 Hz, 1H), 8.09 (t, *J* = 7.4 Hz, 1H), 7.89 (d, *J* = 8.1 Hz, 1H), 7.57 (t, *J* = 9.2 Hz, 1H), 6.95 (d, *J* = 8.7 Hz, 2H), 6.89 (d, *J* = 8.7 Hz, 2H), 6.06 (s, 1H), 5.04 (s, 2H), 3.03 (d, 3.6 Hz 4H), 2.22–2.31 (m, 2H), 1.00 (d, *J* = 6.5 Hz, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 208.11, 194.0, 171.4, 169.4, 166.0, 158.0, 137.4, 129.1, 128.7, 128.5, 128.2, 128.0, 115.3, 107.2, 105.6, 104.3, 70.6, 53.9, 52.6, 32.5, 25.6, 23.3; CIMS: *m/z* 433.17, 238.1 $[M-C_{12}H_{13}O_5]$. Anal. Calcd for $C_{38}H_{38}O_{11}$ (670.70): C, 68.05; H, 5.71. Found: C, 67.22; H, 6.05.

4.4. Cell cytotoxicity assay using MTT

Cytotoxicity of potential candidates was assayed using MTT Kit (Roche, Germany) in the CEM-GFP cell line, according to the manufacturer's protocol. Briefly, 2×10^4 cells/well were seeded in 96-well plate; samples were then added into the wells at different concentrations keeping untreated or vehicle treated wells as controls. After 48–72 h incubation, 10 μL of MTT reagent (5 mg/mL) were added in to the wells to allow the reaction. Formazan crystals produced during the reaction were solubilized and color development was read at 540 nm using microplate ELISA reader.[26]

4.5. Anti-HIV screening in CEM-GFP cells

Human CD4+ T cell line, CEM-GFP cells were infected with HIV-1 $NL_{4.3}$ virus at a multiplicity of infection (MOI) of 0.05 using the standard protocol previously described from the laboratory.[28] The infection was monitored by GFP visualization under a fluorescence microscope. The cells were then incubated with samples for up to 9 days post infection. Preliminary screening was performed by GFP quantitation by microfluorometry. Virus production was assayed in the culture supernatant on day-7 post infection by p24 antigen capture ELISA assay (Perkin–Elmer, USA).

4.6. In vitro HIV-1 reverse transcriptase assay

The reverse transcriptase assay was performed with pure HIV-1 reverse transcriptase using the colorimetric enzyme immunoassay kit obtained from Roche, Germany, following manufacturer's protocol. Nevirapine, a potent non-nucleoside RT inhibitor was used as a positive control.

Acknowledgment

Authors thank Department of Biotechnology, Ministry of Science and Technology, Government of India for the financial support to this project (Grant No. BT/PR7020/Med/14/930/2005).

References and notes

1. Coffin, J. M. *Science* **1995**, *267*, 483.
2. *AIDS Epidemic Update* UNAIDS (http://www.unaids.org) 2009.
3. Dayam, R.; Gundla, R.; Mawsawi, L. Q.-A.; Neamati, N. *Med. Res. Rev.* **2008**, *28*, 118.
4. Clercq, E. D. *Int J Antimicrob. Agents* **2009**, *33*, 307.
5. Donglei, Y.; Susan, M. N.; Kuo, H. L. *Med. Res. Rev.* **2007**, *27*, 108.
6. Singh, I. P.; Bharate, S. B.; Bhutani, K. K. *Curr. Sci.* **2005**, *89*, 269.
7. (a) Buckheit, R. W., Jr.; White, E. L.; Fliakas-Boltz, V.; Russell, J.; Stup, T. L.; Kinjerski, T. L.; Osterling, M. C.; Weigand, A.; Bader, J. P. *Antimicrob. Agents Chemother.* **1999**, *43*, 1827; (b) Kim, H. J.; Yu, Y. G.; Park, H.; Lee, Y. S. *Planta. Med.* **2002**, *68*, 1034; (c) Nishizawa, M.; Emura, M.; Kan, Y.; Yamada, H.; Ogawa, K.; Hamanaka, N. *Tetrahedron Lett.* **1992**, *33*, 2983; (d) Fujoka, T.; Kashiwada, Y. *J. Nat. Prod.* **1994**, *57*, 243; (e) Robinson, W. E.; Reinecket, M. G.; Malekt, S. A.; Jiat, Q.; Chow, S. A. *Proc. Nati. Acad. Sci. U.S.A.* **1996**, *93*, 6326; (f) Saklani, A.; Kutty, S. K. *Drug Discovery Today* **2008**, *13*, 161.
8. Singh, I. P.; Bharate, S. B. *Nat. Prod. Rep.* **2006**, *23*, 558.
9. Nakane, H.; Arisawa, M.; Fujita, A.; Koshimura, S.; Ono, K. *FEBS Lett.* **1991**, 286.
10. Artan, M.; Li, Y.; Karadeniz, F.; Lee, S.-H.; Kim, M.-M.; Kim, S.-K. *Bioorg. Med. Chem.* **2008**, *16*, 7921.
11. Nishizawa, M.; Emura, M.; Kan, Y.; Yamada, H.; Ojima, K.; Hamanaka, M. *Tetrahedron Lett.* **1992**, *33*, 2983.
12. Bokesch, H. R.; Groweiss, A.; McKee, T. C.; Boyd, M. R. *J. Nat. Prod.* **1999**, *62*, 1197.

13. Appendino, G.; Ottino, M.; Marquez, N.; Bianchi, F.; Giana, A.; Ballero, M.; Sterner, O.; Fiebich, B. L.; Munoz, E. *J. Nat. Prod.* **2007**, *70*, 608.
14. Penttila, A.; Kapadia, G. J.; Fales, H. M. *J. Am. Chem. Soc.* **1965**, *87*, 4402.
15. Bharate, S. B.; Bhutani, K. K.; Khan, S. I.; Tekwani, B. L.; Jacob, M. R.; Khan, I. A.; Singh, I. P. *Bioorg. Med. Chem.* **2006**, *14*, 1750.
16. Bharate, S. B.; Khan, S. I.; Yunus, N. A. M.; Chauthe, S. K.; Jacob, M. R.; Tekwani, B. L.; Khan, I. A.; Singh, I. P. *Bioorg. Med. Chem.* **2007**, *15*, 87.
17. Bharate, S. B.; Khan, S. I.; Tekwani, B. L.; Jacob, M.; Khan, I. A.; Singh, I. P. *Bioorg. Med. Chem.* **2008**, *16*, 1328.
18. Marchand, P. A.; Weller, D. M.; Bonsall, R. F. *J. Agric. Food Chem.* **2000**, *48*, 1882.
19. Giorgi, R.; Pifferi, G. European Patent EP 0 348121 A1, 1989; *Chem. Abstr.* **1989**, *113*, 235179.
20. Kuhnke, J.; Bohlmann, F. *Tetrahedron Lett.* **1985**, *26*, 3955.
21. Yao, R. H.; Ma, R. S.; Chen, Y. Q.; Huang, L. S. *Yaoxue Xuebao* **1984**, *19*, 228.
22. Li, L.; Gai, Y. *Huaxue Shiji* **1982**, *4*, 226.
23. Kai, Y. C.; Li, Y.; Yu, P. L.; Cheng, Y. P.; Wang, T. S.; Chen, I. S.; Li, L. C. *Huaxue Xuebao* **1978**, *36*, 143.
24. Yamaki, M.; Miwa, M.; Ishiguro, K.; Takagi, S. *Phytother. Res.* **1994**, *8*, 112.
25. Mitaine-Offer, A. C.; DésiréDjoukeng, J.; Tapondjou, L. A.; Bouda, H.; Lerche, H.; Lontsi, D.; Lacaille-Dubois, M. A. *Biochem. Syst. Ecol.* **2003**, *31*, 1457.
26. Denizot, F.; Rita, L. *J. Immunol. Methods* **1989**, *89*, 271.
27. Gervaix, A.; West, D.; Leoni, L. M.; Richman, D. D.; Wong-Staal, F.; Corbeil, J. *Proc. Natl. Acad. Sci.* **1997**, *94*, 4653.
28. Ladha, J. S.; Tripathy, M. K.; Mitra, D. *Cell Death Differ.* **2005**, *12*, 1417.
29. Mitsuya, H.; Weinhold, K. J.; Furman, P. A.; St Clair, M. H.; Lehrman, S. N.; Gallo, R. C.; Bolognesi, D.; Barry, D. W.; Broder, S. *Proc. Natl. Acad. Sci.* **1985**, *82*, 7096.