NJC

Accepted Manuscript

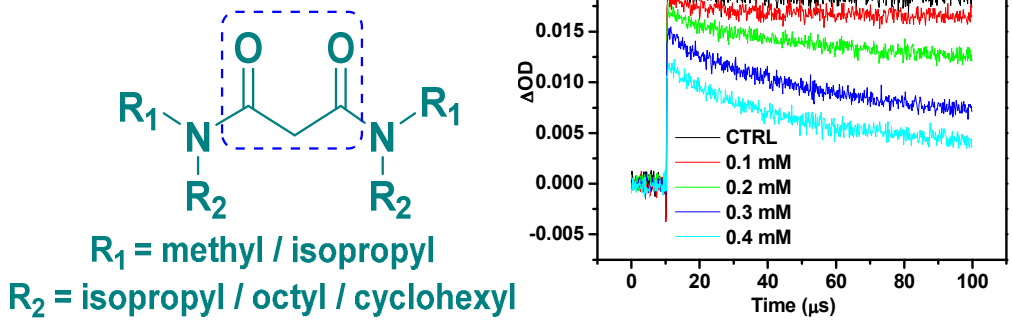
ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/njc

View Article Online
DOI: 10.1039/C4NJ01968G

Evaluation of Malonic acid diamide analogues as radical scavenging agents

Ajay B. Patil[a,c], Sougata Ghosh[b], Suvarna D. Phadatare[e], Priyanath Pathak[c], Geeta K. Sharma[e], Balu. A. Chopade[b,d], and Vaishali S. Shinde[a*]



Radical scavenging ability of malonamides has been explored by use of pulse radiolysis technique.

View Article Online
DOI: 10.1039/C4NJ01968G

Evaluation of Malonic acid diamide analogues as radical scavenging agents

Ajay B. Patil[a,c], Sougata Ghosh[b], Suvarna D. Phadatare[e], Priyanath Pathak[c], Geeta K. Sharma[e], Balu A. Chopade[b,d], and Vaishali S. Shinde[a*]

[a]Garware Research Centre, Department of Chemistry, University of Pune, Pune – 411 007, India.
[b]Institute of Bioinformatics and Biotechnology, University of Pune, Pune – 411 007, India.
[c]Radiochemistry Division, Bhabha Atomic Research Centre, Trombay, Mumbai-400 085, India
[d]Department of Microbiology, University of Pune, Pune – 411 007, India.
[e]National Centre for Free Radical Research, Department of Chemistry, University of Pune, Pune, Pune – 411 007, India.

Abstract

The malonic acid diamide analogues such as *N,N′*-dimethyl-*N,N′*-dioctyl-malonamide (DMDOMA), *N,N′*-dimethyl-*N,N′*-dioctyl-2,(2′-hexyloxyethyl) malonamide (DMDOHEMA), *N,N′*-dimethyl-*N,N′*-dicyclohexyl-malonamide (DMDCMA) and *N,N,N′,N′*-tetraisopropyl malonamide (TiPMA) were synthesized by ester amine coupling method. These synthesized diamides were evaluated for scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, superoxide anion scavenging and ferric reducing antioxidant power (FRAP), by biochemical methods. Antioxidant properties exhibited by these diamides were compared with standard antioxidants like gallic acid and ascorbic acid. Pulse radiolysis technique was employed to generate 2-2′-azinobis 3-ethylbenzothioline-6-sulfonic acid ($ABTS^{\bullet+}$), hydroxyl radical ($^{\bullet}OH$), and carbonate ($CO_3^{\bullet-}$) radicals for evaluating the scavenging activity of the diamides. The DMDCMA, TiPMA, DMDOMA were found to show better antioxidant potential as compared to DMDOHEMA due to more hydrophilic nature. Pulse radiolysis technique was found to be advantageous to know the interaction of the diamides class of compounds with radicals generated under high energy radiations.

Key words: *diamides, pulse radiolysis, antioxidants, metal chelation, free radical scavenging*

*Author to whom correspondence should be addressed;
*E-mail: vsshinde@chem.unipune.ac.in Fax: 91-20-25601395

View Article Online
DOI: 10.1039/C4NJ01968G

1. Introduction

Accumulation of free radicals at high level in cells causes oxidative stress. Reactive oxygen species (ROS) such as superoxide ($O_2^{\bullet-}$), hydroxyl ($^{\bullet}OH$), and peroxyl ($^{\bullet}OOR$) are responsible for oxidative damage to lipid, protein and DNA molecules. This causes development of diseases such as cancer, rheumatoid arthritis, ageing, heart diseases and Alzheimer's disease.[1-3] Antioxidants act as radical scavengers, which react with reactive oxygen species (ROS) and lower the risk of cancer and degenerative diseases.[4] Metal-chelating capabilities of earlier reported antioxidants have enabled them as effective radical scavengers and inhibitors of lipid peroxidation.[5-9] Another important aspect regarding the antioxidant activity, is the lipophilicity of the compounds. It is well established in literature that the lipophilic compounds exhibit good antioxidant behavior in lipid systems.[10,11]

Recently, our group has focused on the synthesis of malonic acid diamides such as *N,N′*-dimethyl-*N,N′*-dioctyl-malonamide (DMDOMA)(Figure 1(1a)), *N,N′*-dimethyl-*N,N′*-dicyclohexyl-malonamide (DMDCMA) (Figure 1(1c)) and *N,N′*-dimethyl-*N,N′*-dioctyl-2,(2′-hexyloxyethyl) malonamide (DMDOHEMA) (Figure 1(4)); and their application as an extractant for the separation of radioactive actinide metal ions by solvent extraction method, and by using liquid membranes under the different experimental conditions in radioactive waste management.[12-15] During such applications, these ligands undergo radiolytic degradation due to ionizing radiation. Therefore, their interaction with free radicals was considered to be matter of greater interest for us, to get insights of their radiological stability in actinide partitioning.[14]

These compounds along with *N,N,N′,N′*-tetraisopropyl malonamide (TiPMA) (Figure 1(1b)) are important ligand molecules. They form very stable complexes with a wide variety of metal ions. However, in the case of malonamides, the complexation with metal ions would significantly affect the keto-enol equilibrium. If the concentration of malonamides is much

View Article Online
DOI: 10.1039/C4NJ01968G

more than that of radioactive actinide metal ions, the excess metal-free malonamides can scavenge ROS in the solutions.[14] B. S. Jursic has studied the use of malonamide derivatives for control of epileptic seizures.[16] It was discovered that certain malonamides exhibit anticonvulsant activity which is a lay symptom in epilepsy, a disease caused by oxidative stress.[17,18] Malonamide analogues were tested for different biological activities such as κ opioid receptors, DNA transfection, cytotoxicity, oxidative stress, and prostaglandin-H synthase inhibition in recent literature.[19-21] Further, the lower basicity of substituted diamides is expected to make them good drugs candidates for treatment of bone diseases, which could be due to better binding to bone, improved bio-availability and superior therapeutic behavior.[22,23] Thus, they can lead to good therapeutic applications such as the antioxidant agents.[24] Recent reports proposed the good stability of malonamide class of compounds towards ionizing radiations, which provided the impetus to study their ability to scavenge the free radicals.[25] Structure-activity relationship in curcumin type of antioxidants proved that the diketone moiety in their structure was responsible for stabilization of radicals and reduces the oxidative damage and diabetic symptoms.[26,27] Though, Metal-chelating capability is not directly correlated with the radical-scavenging activity. The metal chelation would lead to the inhibition of the Fenton reaction, i.e., the generation of hydroxyl radical. In this context, the radical-scavenging activity itself is also important for efficient antioxidants. Therefore, we have envisioned antioxidant activity by malonamides due to presence of 1,3-diketone skeleton which can undergo tautomerization to enol form easily. To investigate radical scavenging and antioxidant activities, superoxide anion is one of the important species. All these radicals play a key role in many naturally occurring processes, since almost all free radicals generated in the biological systems react rapidly with oxygen causing damage.[28,29]

Pulse radiolysis method is ideal for the selective generation of radicals of interest under appropriate experimental conditions as the rate constants of a variety of reactions are

View Article Online
DOI: 10.1039/C4NJ01968G

well documented in the literature.[30-34] Precise knowledge about the yield of radicals makes the evaluation of kinetics easier and thereby rate constants as well as the extinction coefficients of the transients can be determined with good accuracy. It is reported that pulse radiolysis of malonamides has been carried out for the identification of the possible site of $^{\bullet}$OH attack on *N*-methyl amides which was helpful for the study of radical formation in peptides and proteins.[35]

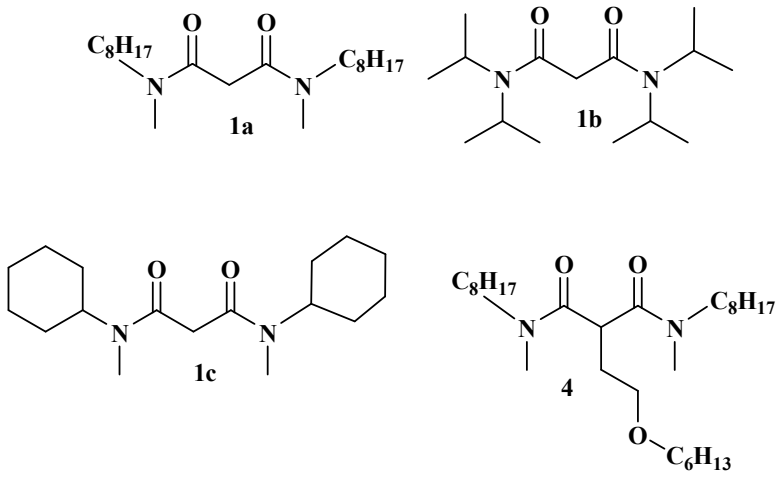


Figure 1. Malonamides used in present work
(1a: DMDOMA, 1b: TiPMA, 1c: DMDCMA, 4: DMDOHEMA)

The present report deals with the radical scavenging studies of DMDOHEMA, DMDOMA, DMDCMA, and TiPMA derivatives (Figure 1).[12,13] The studies towards the antioxidant and radical scavenging activities of synthesized molecules were performed by using pulse radiolysis and biochemical assay techniques. The radical scavenging activities were evaluated by pulse radiolysis for 2-2′-azinobis 3-ethylbenzothioline-6-sulfonic acid ($ABTS^{\bullet+}$), $^{\bullet}OH$, $CO_3^{\bullet-}$ radicals generated by Pune University Linear Accelerator Facility (PULAF). However, 2,2-diphenyl-1-picrylhydrazyl (DPPH), superoxide anion scavenging and ferric reducing antioxidant power (FRAP) study were done by laboratory biochemical methods. The scavenging ability of the synthetic diamides was compared with that of the

View Article Online
DOI: 10.1039/C4NJ01968G

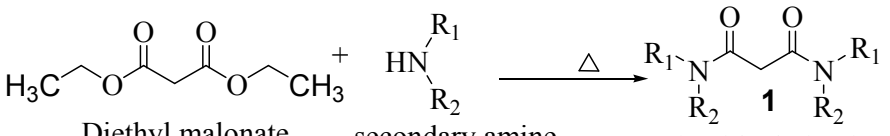
universal antioxidants such as gallic acid and ascorbic acid. The present study was also intended to know the mechanism of interaction of malonamides with the free radicals under high radiation. The set of data thus obtained with different radical solution conditions is correlated for plausible mechanism of radical scavenging by the synthesized diamides.

2. Experimental

2.1. Synthesis of Diamides derivatives:

N-methyl octyl amine, *N*-methyl cyclohexyl amine, and *N,N*-diisopropyl amine (synthesis grade) procured from Alfa Aesar, Fluka, and spectrochem chemicals respectively and was freshly distilled before use. Diethyl malonate (Synthesis grade), ethylene glycol (AR grade), 1-bromohexane (Puriss Grade), dichloromethane (DCM) (AR grade), dimethyl formamide (DMF) (AR grade), sodium hydride (60% suspension in mineral oil) were supplied by Spectrochem Pvt. Ltd., Mumbai, India. IR spectra were recorded using Shimadzu FT-IR spectrometer. NMR spectra were recorded on Varian 300MHz spectrometer. MS analysis was performed on Shimadzu GC-MS Spectrometer.

We have synthesized four maloamide derivatives, *N,N′*-dimethyl-*N,N′*-dioctyl-malonamide (DMDOMA), *N,N′*-dimethyl-*N,N′*-dioctyl-2,(2′-hexyloxyethyl) malonamide (DMDOHEMA), *N,N′*-dimethyl-*N,N′*-dicyclohexyl-malonamide (DMDCMA), *N,N,N′,N′*-tetraisopropyl malonamide (TiPMA) by known literature method[12,13] which is mentioned briefly as: Ester - amine coupling strategy was used for getting the diamide backbone (for DMDOMA, DMDCMA, TiPMA) (Scheme 1).



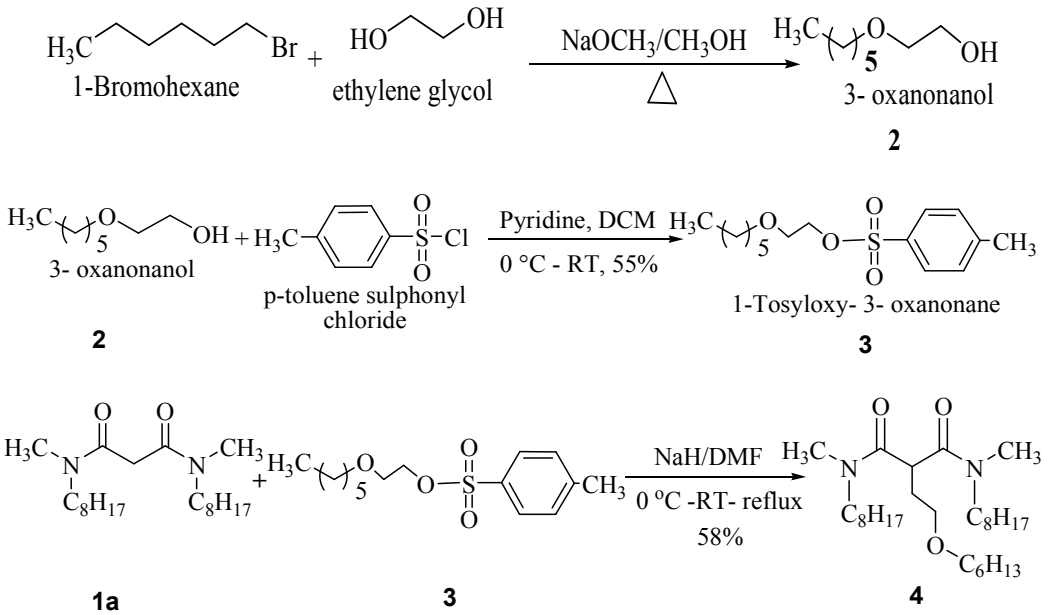
Diethyl malonate | secondary amine | Diamide derivative

1a: DMDOMA; R_1= methyl, R_2= octyl
1b: TiPrMA; R_1= R_2= Isopropyl
1c: DMDCMA; R_1= methyl, R_2= Cyclohexyl

Scheme: 1

View Article Online
DOI: 10.1039/C4NJ01968G

For synthesis of DMDOHEMA (4), the oxyalkyl skeleton (2) was developed by the selective monoprotection of the ethylene glycol using hexyl bromide. It was tosylated (3) to facilitate alkylation to diamide backbone by the NaH (Scheme 2).



Scheme: 2

The synthesized compounds were characterized by analytical techniques such as IR, NMR, MS, etc. The characterization details are as follows:

The yield after column purification was nearly 65-70% for all derivatives.

Characterization for DMDOMA (1a): IR ν_{max}/cm^{-1} :1641cm^{-1}(CO); **^{1}H NMR** (300MHz, $CDCl_3$): δ 0.9(t, 6H), 1.24(br s, 24H), 2.9, 3.1 (t, 6H), 3.7 (m, 4H) 3.45 (d, 2H); **^{13}C NMR** (75MHz, $CDCl_3$): δ 48, 51(*N* - $\underline{C}H_3$), 167 (-$\underline{C}$O-); **MS *m/z*** $C_{21}H_{42}N_2O_2$: **cal** 354.32, **found** 354.

Characterization for TiPMA (1b): IR ν_{max}/cm^{-1} :1645cm^{-1}(CO); **^{1}H NMR** (300MHz, $CDCl_3$): δ 1.2(d, 24H, -N-CH-$\underline{CH_3}$), 3.1(s, 2H, -CO-CH_2-CO-), 3.94 (m, 4H, -N-CH-); **^{13}C NMR** (75MHz, $CDCl_3$): δ 45(*N* - $\underline{C}$H), 165 (-$\underline{C}$O-); **MS *m/z*** $C_{15}H_{32}N_2O_2$: **cal** 270.41, **found** 270.

View Article Online
DOI: 10.1039/C4NJ01968G

Characterization DMDCMA (1c): IR ν_{max}/cm^{-1} :1643cm^{-1}(CO); **^{1}H NMR** (300MHz, $CDCl_3$): δ 1.32–1.84 (20H, multiplet, 2 -CH_2 cyclohexyl), 2.81–2.92 (6H, double doublet, 2 CH_3-N), 3.47–3.51 (2H, triplet, CO-CH_2-CO), 4.40–4.42 (2H, multiplet, 2 -CH cyclohexyl); **^{13}C NMR** (75MHz, $CDCl_3$): δ 52, 56(*N* - $\underline{C}H_3$), 169 (-$\underline{C}$O-); **MS *m/z*** $C_{17}H_{30}N_2O_2$: **cal** 294.43, **found** 294.

Characterization DMDOHEMA (1(4)): IR ν_{max}/cm^{-1}: 1651cm^{-1}(CO). **^{1}H NMR** (300MHz, $CDCl_3$): δ 0.85(t, 9H), 1.2-1.3(br s, 26H), 1.56 (br s, 8H), 2.9 (t, 6H), 3.4 (m, 8H), 3.9 (m, 1H). **^{13}C-NMR** (75MHz, $CDCl_3$): δ 49, 50 (N - $\underline{C}H_3$), 69, 71(-O-$\underline{C}H_2$-),170 (-$\underline{C}$O-). **MS *m/z*** $C_{29}H_{58}N_2O_3$: **cal** 482.78, **found** 483.

2.2. Pharmacology

Folin–Ciocalteu reagent and quercetin was obtained from Qualigens, Mumbai, India. Gallic acid, L-ascorbic acid, potassium thiocyanate, 2-2'-azinobis-3-ethylbenzothioline-6-sulfonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-[Tri(2-pyridyl)-s-triazine] (TPTZ), phenazine methosulfate (PMS), nitroblue tetrazolium (NBT), 2-deoxyribose, thiobarbituric acid (TBA), sodium nitroprusside, sulphanilic acid, *N*-(1-Naphthyl) ethylenediamine dihydrochloride, potassium hexacyanoferrate ($K_3Fe(CN)_6$), trichloroacetic acid (TCA), ferric chloride were procured from HiMedia Laboratories, Mumbai, India.

2.2.1 Pulse radiolysis study

The linear accelerator (LINAC) is an evacuated cylindrical pipe that functions as a waveguide for the accelerating field. The electrons are injected in pulses into this straight segmented waveguide (microwave cavities) and accelerated by the oscillating polarity of the electric field of an electromagnetic wave that travels down the waveguide.[30] High-energy electrons in the range 7MeV can be easily obtained using LINAC, and such high energy

View Article Online
DOI: 10.1039/C4NJ01968G

provides more penetrating power to the electrons and also a uniform distribution of the ionization events throughout the sample.[31] In the present work the pulse radiolysis was done by using Linear accelerator (LINAC) electron pulse radiolysis system at the 'National Centre for Free Radical Research', University of Pune, Pune.[32]

2.2.1.1 Radiolysis generated $ABTS^{\bullet+}$ and carbonate ($CO_3^{\bullet-}$) radical scavenging assay

Scavenging of $ABTS^{\bullet+}$ radical by diamides were determined using pulse radiolysis. The reaction mixture (4 mL) contained 0.05 M sodium azide (NaN_3), 2 mM 2-2'-azinobis 3-ethylbenzothioline-6-sulfonic acid (ABTS) and mili Q water. After purging with N_2O for 5 min, suprasil quartz cuvettes containing the samples were exposed to an electron beam of 100 ns pulse width and dose/pulse 17 Gy generated hydroxyl radicals. The $ABTS^{\bullet+}$ radical was produced by the reaction of radiolytically generated azide radicals with $ABTS^{2-}$. Scavenging of the radical was estimated by recording the traces at 600 nm.[34]

The $CO_3^{\bullet-}$ radicals were generated using a reaction mixture containing 0.5 M $NaHCO_3$ and 0.5 M Na_2CO_3 saturated with N_2O for 5 min in presence of diamides followed by an exposure to an electron pulse of 100 ns. The absorbance was recorded at 600 nm.[28]

2.2.1.2 Hydroxyl radical assay

Hydroxyl radicals ($^{\bullet}OH$) were generated by radiolysis of water using LINAC.[34] Water when exposed to 7 MeV electron pulse generates hydroxyl radicals, hydrated electrons and hydrogen atoms. In order to generate selectively the $^{\bullet}OH$, all solutions were pre-saturated with nitrous oxide (N_2O) for removal of dissolved oxygen. Generated hydroxyl radicals were subjected to react with malonamide derivatives. The first order rate constants (k_1 = s^{-1}) for radical formation were measured and found to vary with the malonamide derivatives. The second order rate constants (k_2 = mol^{-1} dm^3 s^{-1}) were obtained from the slope of linear plots.

View Article Online
DOI: 10.1039/C4NJ01968G

The ability to scavenge hydroxyl radicals was measured by comparing it with standard potassium thiocyanate (KSCN) using competition kinetics. In this method, $^{\bullet}OH$ reacts with 1 mM KSCN in absence and presence of malonamide derivatives. The $^{\bullet}OH$ reacts completely with SCN^{-} to produce $(SCN)_2^{\bullet-}$ which absorbs at 480 nm.[36,37] The rate constant of $(SCN)_2^{\bullet-}$ formation reaction with test malonamide compounds were measured at 480 nm.

2.2.2 Chemical assay for antioxidant activity

2.2.2.1 DPPH radical scavenging assay

Test compounds (20 µL) at concentration of 1 mM to 100 mM were mixed with ethanolic solution (80 µL) of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in 96 well plate. Changes in absorbance were measured at 517 nm (after incubation for 20 min) in a 96-well plate reader (Spectramax M5, Molecular devices corporation, Sunnyvale, CA). Radical scavenging activity was expressed in terms of IC_{50} in mM. Vitamin C (L-ascorbic acid) was served as a reference antioxidant compound.[38]

2.2.2.2 Superoxide anion scavenging activity assay

Superoxide anions were generated in a non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system through the reaction of PMS, NADH, and oxygen. It was assayed by the reduction of nitroblue tetrazolium (NBT). The 0.3 ml of test malonamide compound solution was added in 3 mL of Tris-HCl buffer (100 mM, pH 7.4) containing 0.75 mL of NBT (300 µM) solution and 0.75 mL of NADH (936 µM) solution. The reaction was initiated by adding 0.75 mL of PMS (120 µM) to the mixture. After 5 min of incubation at room temperature, the absorbance at 560 nm was measured in spectrophotometer.[39]

The superoxide anion scavenging activity was calculated according to the following equation:

View Article Online
DOI: 10.1039/C4NJ01968G

% Inhibition = $[(A_0-A_1) / A_0 \times 100]$,

where, A_0 was the absorbance of the control (blank, without compound) and A_1 was the absorbance in the presence of the compound.[39]

2.2.2.3 Ferric Reducing Antioxidant Property

Ferric reducing antioxidant property (FRAP) assay was estimated according to the protocol of Pulido *et. al.*[40] with some modifications. FRAP solution was prepared freshly by mixing 25 ml of acetate buffer, 2.5 ml of TPTZ solution and 2.5 ml of $FeCl_3.6H_2O$ solution, and then warmed to 37°C before use. The 30 µl of individual compound solutions at different concentrations were allowed to react with 0.9 ml of FRAP solution for 15 min in darkness. The absorbance recorded at 595 nm was used to quantify the activity by extrapolating from the standard calibration curve. The % scavenging was expressed in terms of gallic acid equivalent antioxidant capacity (GAEAC).

3. Results and discussion:

3.1 Synthesis of malonamides:

The synthesis of malonamide derivatives with varying structural modifications and hydrophilic nature has been accomplished using ester amine coupling method. The yield and purity was better than earlier reports (~25-30%).[12,41,42] The synthesis of pentaalkyl derivative has been achieved by selective monoprotection, tosylation and C-alkylation methods. The developed synthetic route can be applied to different tailor made alkyl analogues of malonic acid diamides as radical scavenging motifs.

View Article Online
DOI: 10.1039/C4NJ01968G

3.2 Radical scavenging studies:

3.2.1 Pulse radiolysis:

Using linear accelerator, the different radicals such as $ABTS^{\bullet+}$, $^{\bullet}OH$, and $CO_3^{\bullet-}$ has been generated. The rate constant for the reaction of the any reductant with the free radicals indicates its reactivity towards the free radical. Diamide analogues were found to scavenge the generated radicals at their characteristic wavelength. The transient kinetic analysis showed the decrease in second order rate constant with diamide analogues, indicating the inhibition of radical formation. The appearance of the solution was same indicating the stability of the malonamides under high radiation fields. For all pulse experiments, the optimum concentration of malonamides, suitable to give a proper scavenging was 0.4 mM.

3.2.1.1 Carbonate radical scavenging assay:

Carbonate radicals present in the biological systems significantly contributes to the oxidative damage.[28] Therefore, it is necessary to study such radical species for evaluation of the antioxidant activity of malonamides.

As observed in **Figure 2**, the extent of scavenging of carbonate radicals was increased with concentration of malonamide. The highest activity was obtained at 0.4 mM concentration of the malonamide derivatives. Therefore, further assays have been carried out with this optimized diamides concentration. Among all the malonamide derivatives, DMDOHEMA showed the least scavenging which can be attributed to its least hydrophilic nature in the assay solution.

View Article Online
DOI: 10.1039/C4NJ01968G

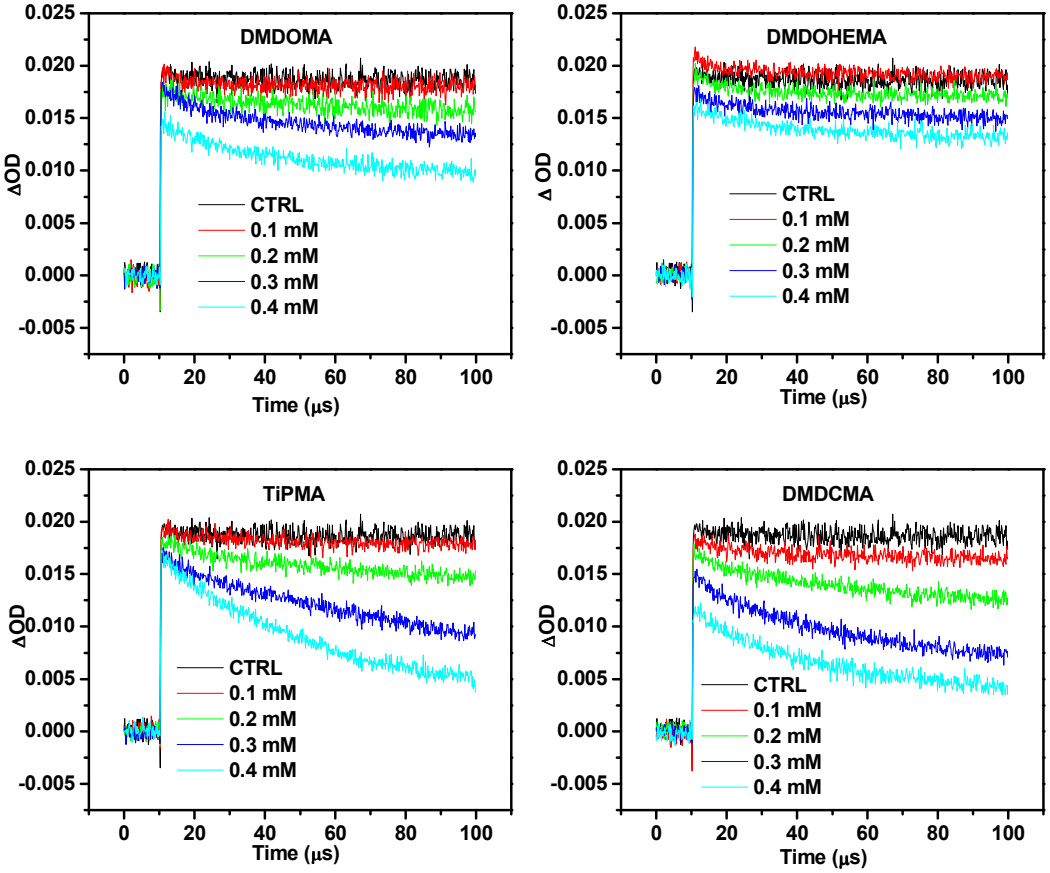


Figure 2: Traces obtained in $CO_3^{\bullet-}$ radical assay by pulse radiolysis at 620 nm using increasing concentration of diamides

3.2.1.2 Hydroxyl radical scavenging assay:

Hydroxyl radical scavenging studies were performed using competition kinetics at 480 nm, using pulse radiolysis. The transient traces were used to determine the second order rate constants as the measure of radical scavenging activity (**Figure 3 and 4**). Malonamides were found to exhibit significantly higher second order rate constant of transient radical as compared to control experiment. DMDOMA and TiPMA were found to be more active in scavenging $^{\bullet}$OH radical than that of compounds DMDOHEMA and DMDCMA due to more hydrophilicity.

View Article Online
DOI: 10.1039/C4NJ01968G

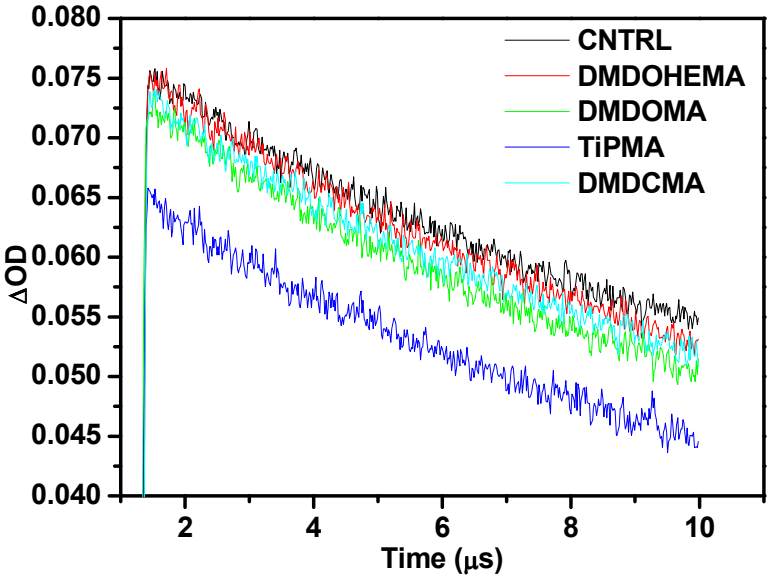


Figure 3: Traces obtained in $^{\bullet}OH$ radical assay by pulse radiolysis at 480 nm using 0.4 mM concentration of diamides

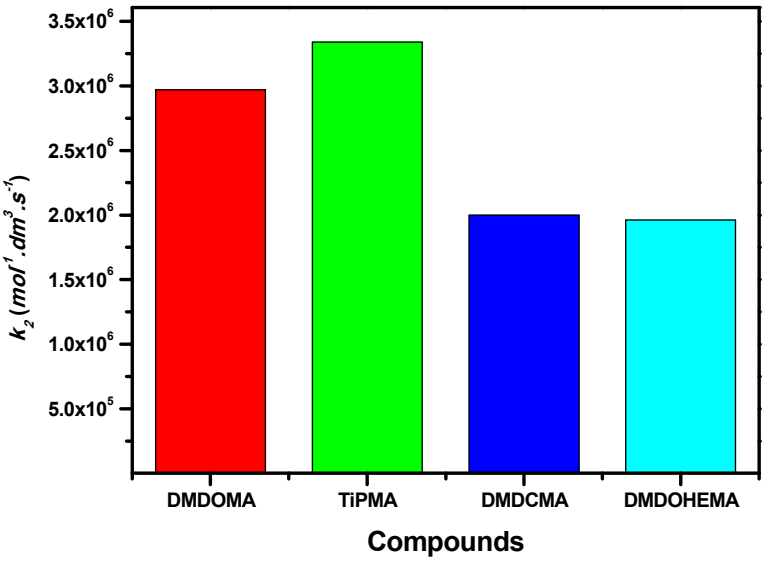


Figure 4: Scavenging of hydroxyl radicals in terms of second order rate constant of different compounds by pulse radiolysis.

3.2.1.3 ABTS radical scavenging assay:

$ABTS^{\bullet+}$ radical was generated using pulse radiolysis technique and easily identified by absorbance at 600 nm. The second order rate constants of transient traces recorded using

View Article Online
DOI: 10.1039/C4NJ01968G

LINAC showed that the extent of radical formation decreased in presence of the malonamide derivatives (**Figure 5**).

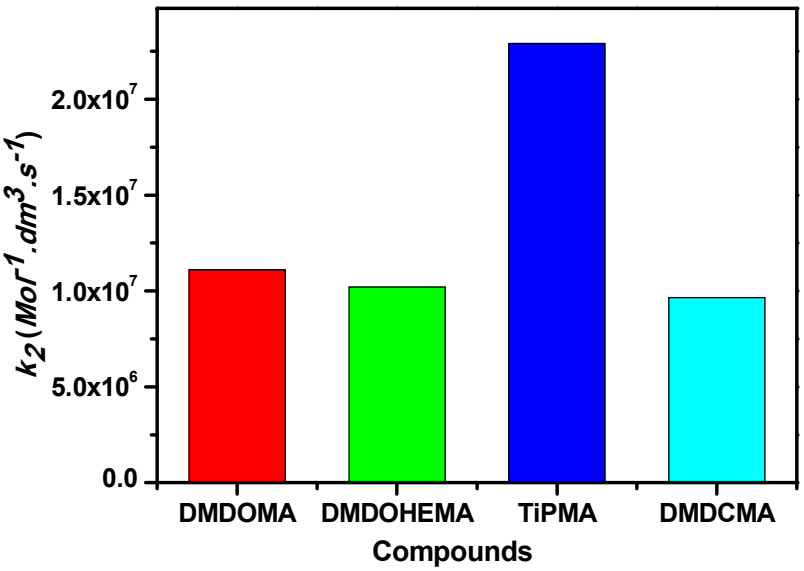


Figure 5: Second order rate constants of traces obtained in ABTS assay by pulse radiolysis at 600 nm using 0.4 mM concentration of diamides

The maximum scavenging efficiency was observed for TiPMA malonamide derivative (second order rate constant, $k_2 = 2.29 \times 10^7$ mol^{-1} dm^3 s^{-1}) while least scavenging was seen for DMDCMA (second order rate constant, $k_2 = 9.62 \times 10^6$ mol^{-1} dm^3 s^{-1}).

3.2.2 Biochemical Assay:

3.2.2.1 DPPH and Superoxide anion scavenging assay:

Malonamides were found to possess comparatively lower scavenging potential against DPPH radical. The least hydrophilic derivative DMDOHEMA showed superior scavenging of DPPH radicals as compared to other counterparts.

A similar trend was observed even in case of the superoxide anion scavenging assay; which is one of the major aspects for the antioxidant evaluation. Therefore, there is a need to study the superoxide anion scavenging activity of the diamides.[28] Superoxide anion scavenging activity

View Article Online
DOI: 10.1039/C4NJ01968G

confirmed the antioxidant properties of the diamide derivatives. Among the malonamides, high % scavenging was found with the DMDOMA and DMDOHEMA (**Table 1**).

Compounds under studies were compared with that of the standard antioxidants such as gallic acid and ascorbic acid. It can be seen that the IC_{50} values for the different malonamide was found to be in similar range with that of gallic acid and ascorbic acid for superoxide anion scavenging activity.

Table 1. Antioxidant activities of malonamide derivatives and their comparison with universal antioxidants

Compound	***Radical Scavenging activity, IC_{50}(mM)***	
	DPPH	***Superoxide anion***
DMDOMA	0.85	0.21
DMDOHEMA	0.62	0.22
TiPMA	0.97	0.27
DMDCMA	1.05	0.24
Gallic Acid	0.61	0.20
Ascorbic Acid	0.64	0.23

3.2.2.2 Ferric ion reducing antioxidant power (FRAP) assay:

Reducing power assay of the antioxidants is based on the reduction of Fe(III) to Fe(II). It was evident that malonamides showed good antioxidant power in the present test solution conditions (**Figure 6**). The FRAP was expressed in terms of gallic acid equivalents (GAEAC) in mM concentration. All four malonamides were compared and found to show good reducing property. Compound TiPMA was showed highest reducing power, while compound DMDOMA showed the least ferric ion reducing antioxidant power.

View Article Online
DOI: 10.1039/C4NJ01968G

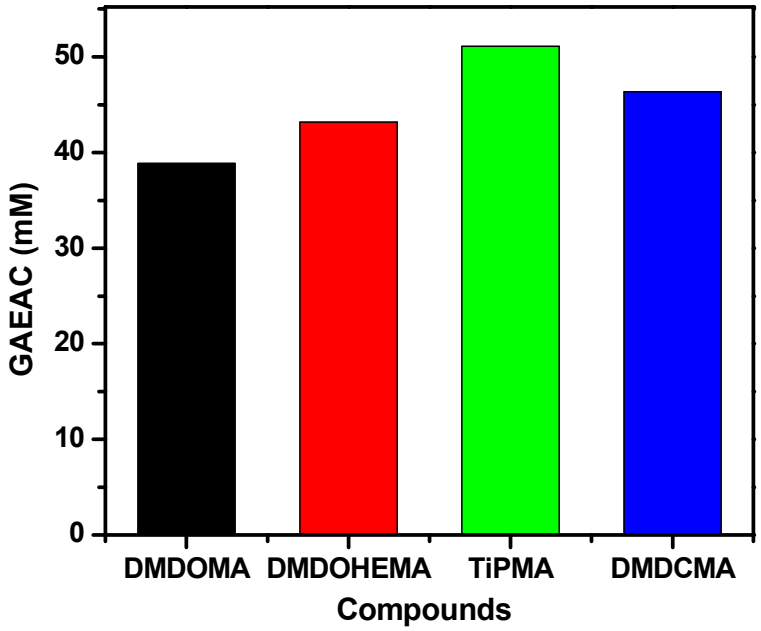


Figure 6: Ferric ion reducing antioxidant power (FRAP) of different malonamide compounds

3.2.3 Plausible radical scavenging mechanism in radical scavenging activity of diamides analogues

From all radical scavenging assays, it is evident that structural relationship of the diamides analogues results in variable scavenging abilities. The least hydrophilic DMDOHEMA possessed least scavenging activity due to presence of the longer alkyl chain substitution on the malonamide skeleton. However, such nonpolar substrates can be exploited as the antioxidant candidates in lipid systems.[10] Also, the diamides polar head serves as the electron rich centre with electron donating nature; which might be the source of scavenging ability as proposed in **Figure 7**.

Similar scavenging mechanism has been proposed earlier for the curcumin's antioxidant activity and can be correlated here as in both the cases, the 1,3-diketone moiety is responsible for radical scavenging.[43] It is already evident that malonamides are present in their tautomeric form, which supports the plausible scavenging pathway showed in **Figure 7**.[12]

View Article Online
DOI: 10.1039/C4NJ01968G

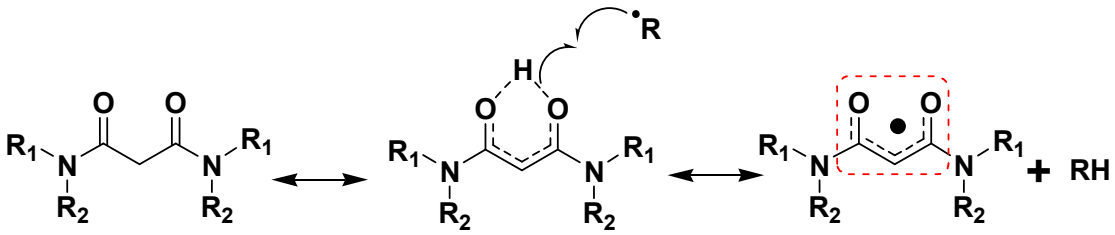


Figure 7: Plausible radical scavenging by malonamide analogues

4. Conclusions

Pulse radiolysis has been applied successfully to study radical scavenging ability of malonamide class of compounds. In present solution conditions, malonamides were found to scavenge carbonate, hydroxyl, ABTS, DPPH radicals and superoxide anion. Metal chelating ability of malonamides has been correlated with radical scavenging ability of malonamide through the plausible tautomerizable moiety. Increasing ability of radical scavenging with less substitution (DMDOHEMA to TiPMA) showed that hydrophilicity is the affecting parameter of scavenging activity of the studied malonamides in the solution conditions. Comparison with the universal antioxidants such as ascorbic acid and gallic acid clearly demonstrated that the malonamides can be good targets for radical scavenging and antioxidant studies. Metal complexes of the said malonamides and lipophilic substituted malonamides can be explored in future as antioxidants for lipid systems.

View Article Online
DOI: 10.1039/C4NJ01968G

5. Acknowledgments

The authors thank Prof. P. K. Mohapatra for helpful discussions. Authors also thank to Prof. A. S. Kumbhar for allowing the use of PULAF facility. ABP great fully acknowledges the financial support for his fellowship from Bhabha Atomic Research Centre, Mumbai, under BARC-University of Pune, Pune Collaborative Research Programme. SG and SDP thanks Council of Scientific and Industrial Research (CSIR, Government of India) for Senior Research fellowship.

View Article Online
DOI: 10.1039/C4NJ01968G

6. References

1. Y. He, P. Yao, S. Chen, Z. Huang, S. Huang, J. Tan, D. Li, L. Gu and Z. Huang, *Eur. J. Med. Chem.*, 2013, 63, 299-312.
2. J.K. Willcox, S.L. Ash and G.L. Catignani, *Crit. Rev. Food Sci. Nutr.*, 2004, 44, 275-295.
3. A. Pe´rez-Gonza´lez, A. Galano and J. Alvarez-Idaboy, *New J. Chem.,* 2014, 38, 2639-2652.
4. L.A. Pham-Huy, H. He and C. Pham-Huy, *Int. J. Biomed. Sci.*, 2008, 4, 89-96.
5. J. Terao, and M.K. Piskula, in Flavonoids as inhibitors of lipid peroxidation in membranes, in C.A. Rice-Evans and L. Packer (editor), *Flavonoids in health and disease*, Marcel Dekker, New York, 1997, p.p. 277-295.
6. N.R. Perron and J.L. Brumaghim, *Cell. Biochem. Biophys.*, 2009, 53, 75–100.
7. S.K. Suthar, V. Jaiswal, S. Lohan, S. Bansal, A. Chaudhary, A. Tiwari, A.T. Alex and A. Joesph, *Eur. J. Med. Chem.* 2013, 63, 589-602.
8. D. Hadjipavlou-Litina, G.E. Magoulas, M. Krokidis and D. Papaioannou, *Eur. J. Med. Chem.*, 2010, 45, 298–310.
9. A. Barik, B. Mishra, A. Kunwar, R.M. Kadam, L. Shen, S. Dutta, S. Padhye, A.K. Satpati, H. Zhang and K.I. Priyadarsini, *Eur. J. Med. Chem.*, 2007, 42, 431-439.
10. L. Liu, C. Jin and Ying Zhang, *RSC Adv.*, 2014, 4, 2879-2891.
11. M. Barontini, R. Bernini, I. Carastro, P. Gentili and A. Romani, *New J.Chem.*, 2014, 38, 809-816.
12. A. B. Patil, V. S. Shinde, P. N. Pathak, P. K. Mohapatra and V. K. Manchanda, *Radiochim. Acta*, 2013, 101, 93-100.
13. A. B. Patil, V. S. Shinde, P. N. Pathak, and P. K. Mohapatra, *Sep. sci. Technol.*, 2014, (In press DOI: 10.1080/01496395.2014.943772).

View Article Online
DOI: 10.1039/C4NJ01968G

14. A. B. Patil, P. N. Pathak, V. S. Shinde, S. V. Godbole and P. K. Mohapatra, *Dalton Trans.*, 2013, 42, 1519-1529.

15. A. B. Patil, P. Kandwal, V. S. Shinde, P. N. Pathak and P. K. Mohapatra, *J. Membra. Sci.*, 2013, 442, 48-56.

16. B. S. Jursic, *Tet. Lett.*, 2005, 41, 325-328.

17. C. M. Darling, US Patent No. 1985, 4 537 781.

18. C. M. Darling and P. Pryor, *In J. Pharm. Sci.*, 1979, 68, 108-110.

19. C. Wolk, M. Heinze, P. Kreideweiß, M. Dittrich, G. Brezesinski, A. Langner and B. Dobner, *Int. J. Pharmaceut.*, 2011, 409, 46–56.

20. G. Chu, M. Gu, J.A. Cassel, S. Belanger, T. M. Graczyk, R.N. DeHaven, N. Conway-James, M. Koblish, P. J. Little, D. L. DeHaven-Hudkins and Roland E. Dolle, *Bioorg. Med. Chem. Lett.*, 2007, 17, 1951–1955.

21. J. L. Vennerstrom and T. J. Holmes, *J. Med. Chem.*, 1987, 30, 434-437.

22. M. M. A. Boojar and A. Shockravi, *Bioorg. Med. Chem.*, 2007, 15, 3437–3444.

23. P. Sozio, E. D'Aurizio, A. Iannitelli, A. Cataldi, S. Zara, F. Cantalamessa, C. Nasuti and A. Di Stefano, *Arch. Pharm. Chem. Life Sci.*, 2010, 343, 133 – 142.

24. D. A. Stepinski and A. W. Herlinger, *Syn. Comm.*, 2002, 32, 2683–2690.

25. L. Berthon, J. M. Morel, N. Zorz, C. Nicol, H. Virelizier and C. Madic, *Sep. Sci. Technol*., 2001, 36, 709-728.

26. U. Singh, A. Barik, B. G. Singh and K. I. Priyadarsini, *Free Rad. Res.*, 2011, 45, 317-325.

27. J. S. Rathee, B. S. Patro, S. Mula, S. Gamre and S. Chattopadhyay, *J. Agric. Food Chem.*, 2006, 54, 9046-9054.

28. D. Huang, B. Ou and R. L. Prior, *J. Agric. Food Chem.*, 2005, 53, 1841-1856.

View Article Online
DOI: 10.1039/C4NJ01968G

29. A. Pérez-González, A. Galano and J. R. Alvarez-Idaboy, *New J. Chem.*, 2014, 38, 2639-2652.

30. P. Yadav, M. S. Kulkarni, M. B. Shirdhonkar and B. S. M. Rao, *Curr. Sci.*, 2007, 92, 599-605.

31. S. D. Phadatare, K. K. Sharma, B. S. M. Rao, S. Naumov and G. K. Sharma, *J. Phys. Chem. B*, 2011, 115, 13650-13658.

32. J. S. Londhe, T. P. A. Devasagayam, L. Y. Foo and S. S. Ghaskadbi, *Redox Report*, 2008, 13, 199-207.

33. R. Joshi, P. N. Pathak, V. K. Manchanda, S. K. Sarkar and T. Mukherjee, *Res. Chem. Intermed.*, 2010, 36, 503–510.

34. J. S. Londhe, T. P. A. Devasagayam, L. Y. Foo and S. S. Ghaskadbi, *J. Radiat. Res.*, 2009, 50, 303–309.

35. S. Rustagi and P. Riesz, *Int. J. Radiat. Biol.*, 1978, 33, 325-339.

36. G. H. Naik, K. I. Priyadarsini and H. Mohan, *Curr Sci.*, 2006, 90, 1100–1105.

37. S. Ghosh, A. Derle, M. Ahire, P. More, S. Jagtap, S. D. Phadatare, A. B. Patil, A. M. Jabgunde, G. K. Sharma, V. S. Shinde, K. Pardesi, D. D. Dhavale and B. A. Chopade, *Plos One*, 2013, 8, e82529.

38. M.A.A. Rahman and S.S. Moon, *Bull. Korean Chem. Soc.*, 2007, 28, 827-831.

39. F. Liu, V. E. C. Ooi and S. T. Chang, *Life Sci.,* 1997, 60**,** 763-771.

40. R. Pulido, L. Bravo and F. Saura-Calixto, *J Agric Food Chem.*, 2000, 48, 3396–3402.

41. E. G. Knapick, P. Ander and J. A. Hirsch, *Synthesis*, 1985, 58-60.

42. G. Thiollet and C. Musikas, *Solv. Extr. Ion Exch.*, 1989, 7, 813-827.

43. K. I. Priyadarsini, *Curr. Pharma. Des.*, 2013, 19, 2093-2100.