Reactive methylene compounds as synthons for various bio active molecules

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Abstract:

Novel malonamic acid, hydrazide and amide were efficiently synthesized from the condensation of 3-NO_2 aniline with diethyl malonate. Also the synthesis of coumarins, azo coumarins. benzocoumarins, cinnamamide, and α:β-unsaturated acid, were achieved by the reaction of above synthesized compounds in a single step reaction in good to excellent yields. And these eight compounds were tested for their antibacterial activities with two bacteria E. coli and S. aureus. Compounds are showing slightly to moderate antibacterial activities against same bacterias.

Key words: Reactive methylene compounds, antibacterial activity**.**

Introduction:

Organic compounds containing the reactive methylene group provide excellent intermediates in synthetic organic chemistry. Such substances have been found to be useful as synthons for various bioactive agents. Using such type of compounds as starting material quiet a large number of heterocyclic and non-heterocyclic compounds can be prepared by condensing them with other substances. Heterocycles form by far the largest of classical divisions of organic chemistry. A broad spectrum of biological activity associated with heterocyclic compounds has attracted interest in drug discovery research. As evident from literature, both synthetic as well as natural oxygen and nitrogen containing heterocyclic molecules possesses significant antimicrobial activities and a large number have been made up to clinics for health care worldwide. Coumarins are classes of heterocycles which exhibits diverse chemical and biological properties[1-15].

And also the non-heterocyclic compounds like cinnamamide, α:β-unsaturated acid, hydrazones and thiosemicarbazides[16-21] have attracted significant interest in medicinal, agricultural and industrial chemistry.

Experimental Section:

Melting points were determined in open capillary tubes and are uncorrected. The purity of the compound was checked on silica-gel-coated Al plates (Merck). The structures of the compounds

are confirmed on the basis of their Infrared spectra (IR) using KBr discs, on a Perkin Elmer Spectrum RX1 infrared spectrophotometer. 1H NMR spectra were recorded in DMSO on Bruker DRX-300 (300 MHz) and Jeol AL300 FT-NMR (300 MHz) systems; chemical shift (δ) is reported in ppm using TMS as an internal reference. Elemental analysis was performed on Elementor Vario EL III. All the compounds gave satisfactory microanalysis.

1. ***3-(3-nitrophenylamino)-3-oxopropanoic acid*** **(I):** To the 3-nitro aniline (0.05 moles, 6.9 gm), diethyl malonate (0.05mol) was added and refluxed for 1-1.30 hr. It was then cooled and filtered. 10 ml ethanol was added to the filtrate and the solution was concentrated on steam bath. Steam was blown into the mixture of above concentrated solution and 20ml ethanol in the presence of 20% Na_2CO_3 solution (10 ml). It was then cooled and filtered. To the filtrate, conc. HCl was added. The cream colored solid thus separated was filtered, washed with water and recrystallized from absolute ethanol.

   Spectral data for compound (I): IR: ν = 737, 1351, 1433, 1486, 1594, 2729, 2823, 3355 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ: 3.63 (s, 2H, CH_2), 7.59-7.94 (m, 4H, Ar-H), 8.61 (s, 1H, CONH), 10.66 (s, 1H, COOH).

2. ***3-(3-nitrophenylamino)-3-oxopropane hydrazide*** **(II):** To the 3-nitro aniline (0.05 moles, 6.9 gm), diethyl malonate (0.05mol) was added and refluxed for 45-60 mins. It was then cooled and filtered. 10 ml ethanol was added to the filtrate and the solution was concentrated on the steam bath. After cooling it to the room temperature, 20 ml ethanol and hydrazine hydrate (0.05 mole, 99%) was added and refluxed for 45 mins on the steam bath. On cooling, white crystalline solid was separated; it was then filtered and recrystallized from absolute ethanol.

   Spectral data for compound (II):. IR: ν = 741, 1012, 1511, 1595, 1629, 2810, 3478 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ: 3.54 (s, 2H, CH_2), 4.30 (s, 2H, NH_2), 5.79 (s, 1H, CONH), 7.59-8.56 (m, 4H, Ar-H), 9.21 (s, 1H, CONH-Ar).

3. ***N^1-(3-nitrophenyl) malonamide*** **(III):** To the 3-nitro aniline (0.05 moles, 6.9 gm), diethyl malonate (0.05mol) was added and refluxed for 45-60 mins. It was then cooled and filtered. 10 ml ethanol was added to the filtrate and the solution was concentrated on the steam bath. After cooling, 10 ml ethanol and 10ml of liquor ammonia (d= 0.88) was added to this concentrated solution, taken in a flask. The flask is tightly corked and shaken vigorously for 1 hr. leaving it overnight, pale yellow crystalline solid was separated; it was then filtered and recrystallized from absolute ethanol.

   Spectral data for compound (III): IR: ν = 1352, 1592, 1689, 2928, 3445 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ: 3.59 (s, 2H, CH_2), 5.79 (s, 2H, NH_2), 7.54-7.95 (m, 4H, Ar-H), 8.64(s, 1H, CONH).

4. ***8-bromo-6-chloro-coumarin-3-carboxy-(3'-nitro) phenyl amide*** **(IV):** A mixture of compound (**I)** (0.001 mole, 0.224 gm), 3-bromo-5-chloro salicylaldehyde (0.001 mole, 0.235 gm) and 2 drops of pyridine was heated in an oil bath at 105-115 ^{o}C for 4 hr. The mixture first melted and later solidified to orange colored mass. After cooling, it was digested with a saturated solution of sodium bicarbonate (10ml) and filtered. The residue was then boiled with 10 ml ethanol and filtered hot. The pale orange solid left was purified by repeated washing with boiling methanol, ethanol and acetone.

   Spectral data for compound (IV):IR: ν = 698, 760, 1255, 1356, 1590, 1720, 3450 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.23-8.74 (m, 6H, Ar-H), 8.09(s, 1H, CONH), 8.24 (s, 1H, CH).

5. ***2-hydroxy naphthyl-coumarin-3-carboxy-(3'-nitro) phenyl amide*** **(V):** A mixture of compound (**I)** (0.001 mole, 0.224 gm), 2-hydroxy-1-naphthaldehyde (0.001 mole, 0.172 gm) and 2 drops of pyridine was heated in an oil bath at 105-115 ^{o}C for 4 hr. The mixture first melted and later solidified to brown colored mass. After cooling, it was digested with a saturated solution of sodium bicarbonate (10ml) and filtered. The residue was then boiled with 10 ml ethanol and filtered hot. The orange solid left was purified by repeated washing with boiling methanol, ethanol and acetone.

   Spectral data for compound (V): IR: ν = 1213, 1351, 1592, 1707, 3464 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 6.93-8.69 (m, 10H, Ar-H), 8.73 (s, 1H, CONH), 8.89 (s, 1H, CH).

6. ***6-(3'-methoxy) phenyl azocoumarin-3-carboxy-(3'-nitro) phenyl amide*** **(VI):** A mixture of compound (**I)** (0.001 mole, 0.224 gm), 2-hydroxy-5-(3-methoxy) phenyl azo benzaldehyde (0.001 mole, 0.260 gm) and 2 drops of pyridine were heated in an oil bath at the 105-115 ^{0}C for 4 hours. The mixture first melted and later solidified to dark brown colored mass. After cooling, it was digested with a saturated solution of sodium bicarbonate (10ml) and filtered. The residue was then boiled with 10 ml ethanol and filtered hot. The brown solid left was purified by repeated washing with boiling methanol, ethanol and acetone.

   Spectral data for compound (VI): IR: ν = 1252, 1351, 1460, 1591, 1711, 2819, 3455 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.81 (s, 3H, OCH_3), 7.20-8.83 (m, 11H, Ar-H), 8.50 (s, 1H, CONH), 8.31 (s, 1H, CH).

7. ***N-(3'-nitro) phenyl-3,4-dimethoxy cinnamamide*** **(VII):** A mixture of compound (**I)** (0.001 mole, 0.224 gm), 3,4-dimethoxy benzaldehyde (0.001 mole, 0.166 gm) and 2 drops of pyridine was heated in an oil bath at 105-115 ^{o}C for 4 hr. The mixture first melted and later solidified to yellowish brown colored mass. After cooling, it was digested with a saturated solution of sodium bicarbonate (10ml) and filtered. The residue

was washed with hot water and recrystallized from aqueous ethanol to give shiny yellow crystals.

Spectral data for compound (VII): IR: ν = 738, 1259, 1515, 1594, 1621, 2819, 3422 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ: 3.81 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 6.67 (s, 1H, CH), 6.71-8.76 (m, 7H, Ar-H), 8.00 (s, 1H, CONH).

8. ***3-(3',4'-dimethoxy benzal)-N-(3'-nitro)phenyl malonamic acid*** **(VIII):** A mixture of compound (**I)** (0.001 mole, 0.224 gm), 3,4-dimethoxy benzaldehyde (0.001 mole, 0.166 gm) was heated in an oil bath at 105-115 ^oC for 4 hr. The mixture first melted and later solidified to dark brown colored mass. After cooling, it was digested with a saturated solution of sodium bicarbonate (10ml) and filtered. To the filtrate, conc. HCl was added. The brown colored precipitate recrystallized from absolute ethanol.

   Spectral data for compound (VIII): IR: ν = 1260, 1352, 1624, 1720, 2825, 3023, 3448 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ: 3.34-3.87 (s, 6H, OCH_3), 6.67 (s, 1H, CH), 6.79-8.73 (m, 7H, Ar-H), 8.62 (s, 1H, CONH), 11.01 (s, 1H, OH).

Antibacterial activity:

A filter paper disc technique using Hi-Media agar medium is employed to study the antibacterial activity of compounds (I-VIII) against Gram positive bacteria Staphylococcus aureus (S. aureus) and Gram negative bacteria Escherichia coli (E.coli). The concentration of test compounds is 1,000µg/ml. After 48 hr incubation at 37 ^oC, zone of inhibition produced by each compound is measured in mm as shown in Table 1. Streptomycin is used as the reference drug and Dimethyl formamide as a control.

Table- 2 Antibacterial activity of the compounds I-VIII

Key to symbols: Resistance = R; slightly active = + (inhibition zone 6-9mm); moderately active = + + (inhibition zone 9-12 mm); highly active = + + + (inhibition zone> 12 mm).

Result and Discussion:

To gain access to a good amount of novel malanomic acid (**I**), hydrazide (**II**) and amide (**III**), we studied the reaction of commercially available 3-NO_2 aniline with freshly distilled Diethyl malonate. Diethyl malonate and 3-nitro aniline was refluxed in equimolar proportion. It was then cooled and concentrated. Steam was blown into the mixture of above concentrated liquid and 20ml ethanol in the presence of 20% Na_2CO_3 solution. Cooling and decomposition with concentrated HCl furnished N-(3-nitro) phenyl malonamic acid (**I**). N-(3-nitro) phenyl malonamic acid hydrazide (**II**) and N-(3-nitro) phenyl malonamide (**III**) was obtained by treating the above concentrated liquid with hydrazine hydrate (99%) and ammonia (d=0.88) respectively. The chemical reaction proceeds as described in **Scheme A.** Our next concern was directed towards the reaction of a compound **(I)** with different substituted aldehydes with

catalyst and without catalyst. Compound **(I)** undergoes condensation with commercially available 3-bromo-5-chloro salicylaldehyde and 2-hydroxy-1-naphthaldehyde to yield coumarin (IV) and benzo coumarin (V) respectively in the presence of 2 drops of pyridine. Azocoumarin (VI) was also synthesized by condensing of compound (I) in presence of catalyst with 2-hydroxy-5-(3-methoxy) phenyl azo benzaldehyde, which was prepared by the reaction of salicylaldehyde with diazotized amine[22]. Compound (I) when condensed with 3,4-dimethoxy benzaldehyde in the presence of catalyst furnished cinnamamide (VII) while the same in the absence of catalyst resulted into corresponding α:β-unsaturated acid (VIII). The chemical reactions are summarized in **Scheme B**. All synthesized compounds were tested against gram positive and gram negative bacteria by using the filter paper disc technique.

Conclusion:

We develop novel, versatile and efficient method for the synthesis of bioactive coumarins, azocoumarins, benzocoumarins, cinnamamide, and α:β-unsaturated acid, we herein report efficient synthesis of these candidates from reactive methylene compounds of malonamic acid series. Antibacterial activities of all the synthesized compounds were performed against one gram negative bacteria (E.coli) and one gram positive bacteria (S.aureus). All tested compounds except **VIII**, which is inactive against both bacteria showed slight to moderate antibacterial activities. Compounds IV & VI showed moderate biological activity against same bacteria's. And the compound III, V, & VII showed resistance to E. coli bacteria. To the best of our knowledge, this is the report on the synthesis of the above mentioned compounds from economical and easily available diethyl malonate and primary aromatic amine.

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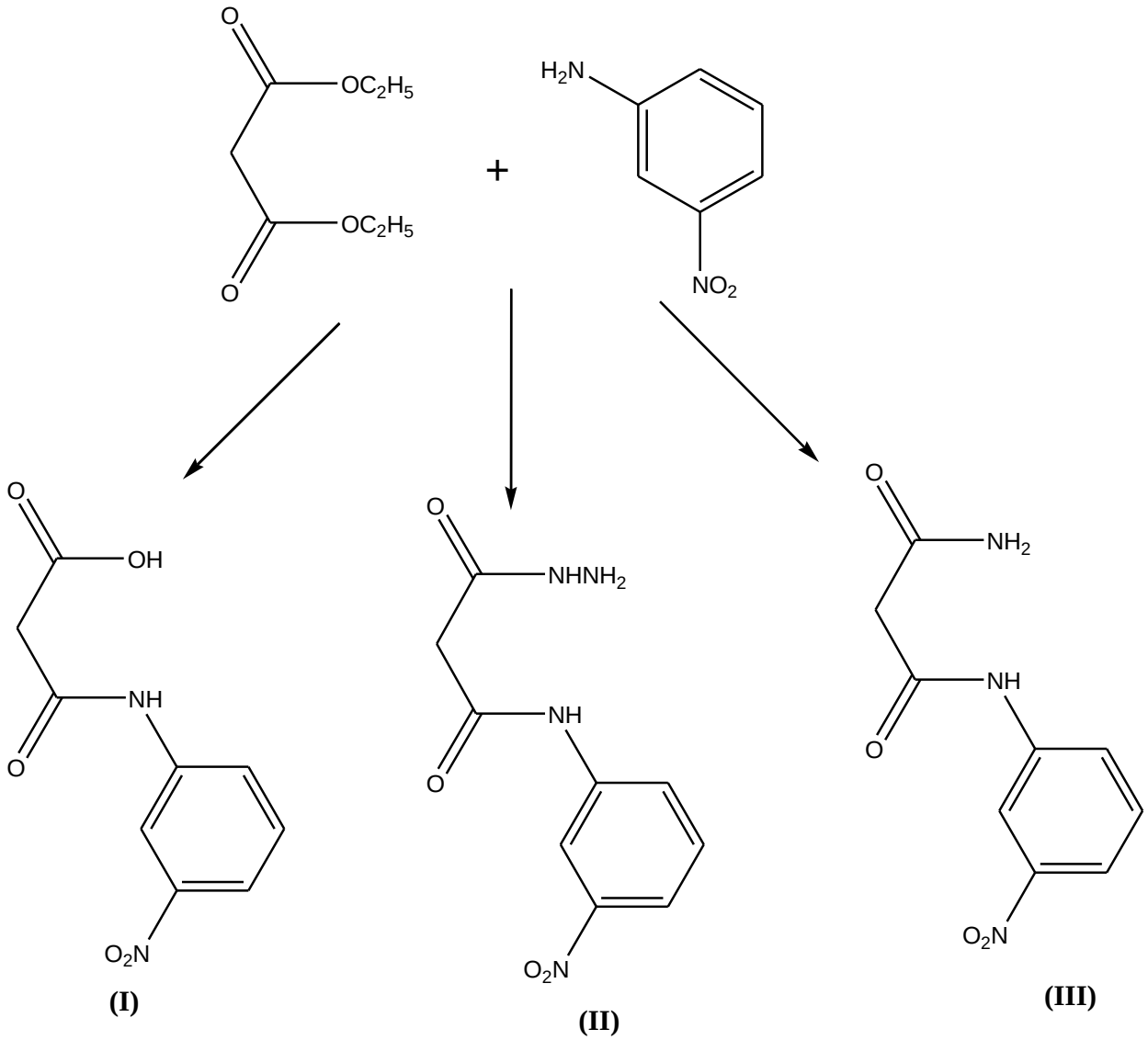
O
OC2H5
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+
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(I)
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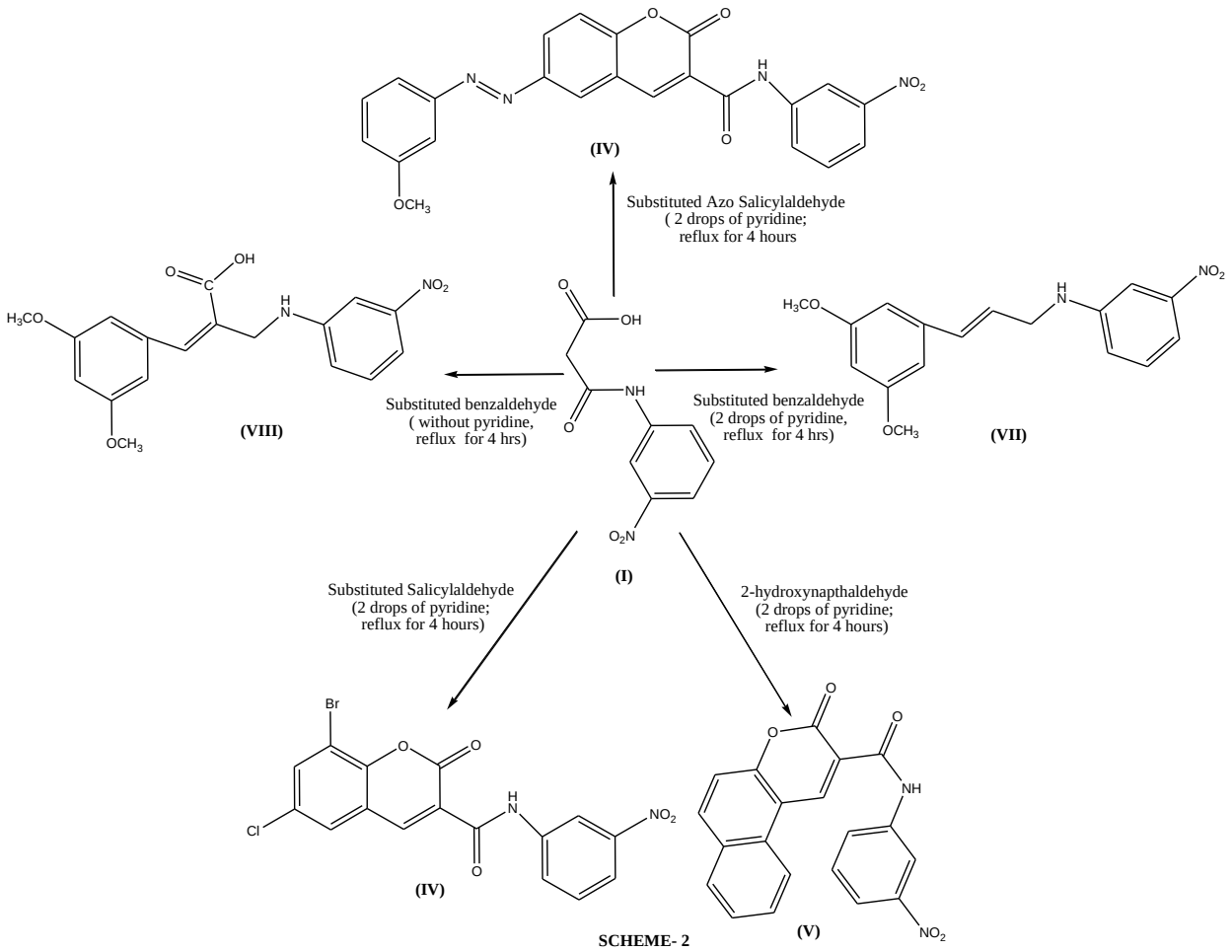
SCHEME-1



SCHEME- 2

Table-1 Physical and analytical data of synthesized compounds

S. N 0.	Compd	M.P (^{0}C)	% Yield	Mol. Formula	% Carbon		% Hydrogen		% Nitrogen	
					Found	Calc.	Found	Calc.	Found	Calc.
1	**(I)**	120 -122	71.11	$C_9H_8O_5N_2$	48.30	48.21	3.55	3.57	12.53	12.50
2	**(II)**	152 -154	85.96	$C_9H_{10}O_4N_4$	45.40	45.38	4.18	4.20	23.55	23.53
3	**(III)**	178 -180	70.14	$C_9H_9O_4N_3$	48.42	48.43	4.05	4.04	18.90	18.83
4	**(IV)**	200 -201	60.46	$C_{16}H_8O_5N_2$ ClBr	45.46	45.44	1.88	1.88	6.60	6.60
5	**(V)**	>300	65.71	$C_{20}H_{12}O_5N_2$	66.64	66.67	3.30	3.33	7.75	7.78
6	**(VI)**	>300	77.27	$C_{23}H_{16}O_6N_4$	62.15	62.16	3.55	3.60	12.60	12.61
7	**(VII)**	203	65.63	$C_{17}H_{16}O_5N_2$	62.07	62.19	4.80	4.88	8.55	8.54
8	**(VIII)**	102 -104	32.43	$C_{18}H_{16}O_7N_2$	58.10	58.06	4.25	4.30	7.50	7.53

Table- 2. Antibacterial activity of compounds against two bacterial strains

Compound N0.	E. coli	S. aureus
I	+	+
II	+ +	+
III	+	R
IV	+ +	+ +
V	+	R
VI	++	+ +
VII	+	R
VIII	R	R
Streptomycin	+ + +	+ + +

Key to symbols: Resistance = R; slightly active = + (inhibition zone 6-9mm); moderately active = + + (inhibition zone 9-12 mm); highly active = + + + (inhibition zone> 12 mm). MIC expressed in mm.

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