Available online at www.joac.info

ISSN: 2278-1862

Journal of Applicable Chemistry

2014, 3 (1): 47-55

(International Peer Reviewed Journal)

Synthesis, Characterization and Pharmacological study of Metacetamol derivatives

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Accepted on 27th December 2013

ABSTRACT

A series of new metacetamol derivatives were synthesized from 3-Aminophenol which was prepared from 3-nitrophenol in the presence of palladium carbon in hydrogen atmosphere. The compound was treated with acetic anhydride, and was further alkylated with different substituted Benzyl bromides to yield alkylated metacetamol derivatives. The synthesized compounds were characterized by LC-MS, ^{1}H NMR, and ^{13}C NMR and IR spectral studies. The compounds were screened for antibacterial and anthelmintic activity. Three of the compounds showed moderate antibacterial and anthelmintic activity compared to standard ciprofloxacin drug.

Keywords: Metacetamol, m-nitrophenol, acetic anhydride, substituted Benzyl bromides, antibacterial activity, anthelmintic activity

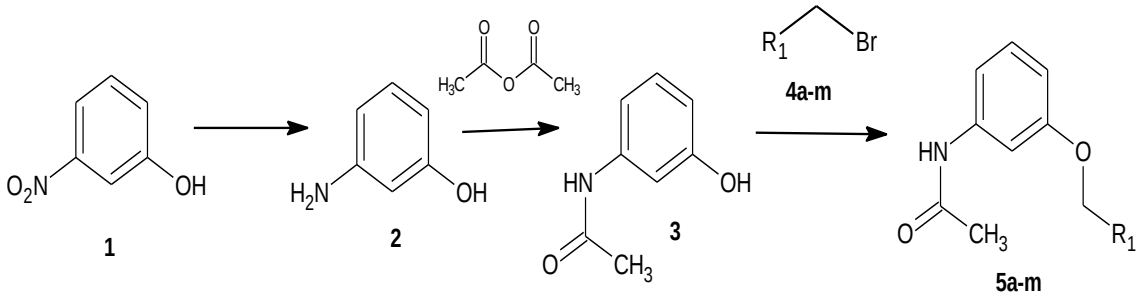
INTRODUCTION

Paracetamol is a commonly used analgesic and antipyretic drug and is being used since more than six decades. It has been proved to be an excellent and effective drug for pain relief and control of fever in adults and children [1]. Recently Paracetamol derivatives were found to possess significant biological activities such as antibacterial [2-4], anti-inflammatory [5], analgesic, antipyretic [6, 7] activities. In addition, a few condensed paracetomol derivatives were found to possess bronchodilator effect [8] and antitubercular activities [9]. In literature, reviews on alkylation reactions [10-12], formulations [13, 14] and dosage [15, 16] are well documented. Therefore, there has been an impetus in search for discovery and development of newer pharmacologically active paracetamol derivatives. Hence we made an attempt to synthesize novel Metacetamol derivatives and to evaluate their antibacterial and anthelmintic activities.

MATERIALS AND METHODS

Melting points reported were determined in open capillary and are uncorrected. The newly synthesized compound structures were established using IR, ^{1}H NMR, ^{13}C NMR and LC-MS data. FT-IR Spectra was recorded on Jasco FT-IR Spectrometer, ^{1}H NMR and ^{13}C NMR were recorded in Bruker model avance II (399.65 MHz, ^{1}H NMR) and Bruker model avance II (100.50 MHz, ^{13}C NMR) instruments respectively, analysis were carried out either DMSO-d_6 or $CDCl_3$ depending on solubility of the compound. All the chemical shifts were reported in parts per million (ppm). LC-MS was recorded using Waters Alliance 2795 separations module and Waters Micro mass LCT mass detector. Elemental analysis (C, H and N) was performed on a Elementar vario MICRO cube. The purity of the compound was confirmed using TLC on precoated silica gel plate and further purification was done using column chromatography.

Experimental: Synthetic route for preparation of Metacetamol derivatives is shown in **scheme 1**



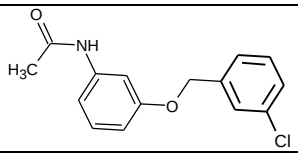
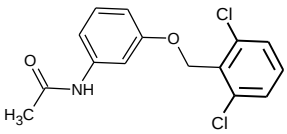
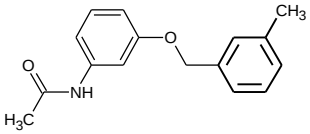
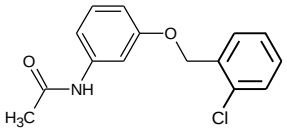
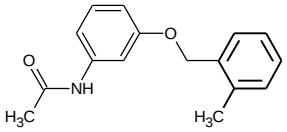
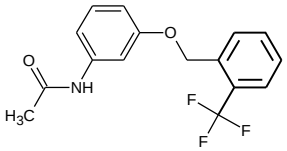
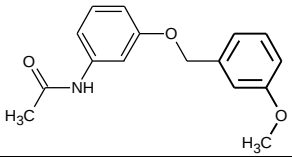
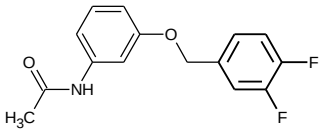
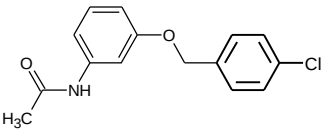
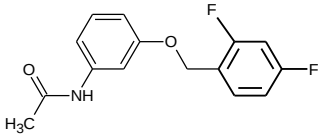
Scheme 1: Synthesis of metacetamol derivatives

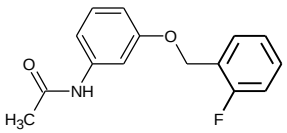
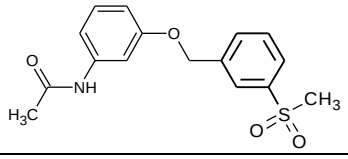
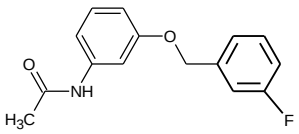
Procedure for the preparation of 3-aminophenol (2): A mixture of 3-nitrophenol **(1)** (0.13 mol, 25 g), and palladium carbon (Pd/C) (10 % by wt, 2.5 g) in 250 mL of dry methanol was stirred for 10 h at 2 kg pressure at room temperature. The reaction was monitored by TLC, the Pd/C was filtered, methanol was removed under vacuum, and the residue was dissolved in MDC, washed with water. The organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated. The crude compound was obtained by triturating the concentrated mass with petroleum ether and diethyl ether. It was filtered and dried to get title compound as a white solid. The product was identified by melting point and confirmed from spectral studies. LCMS: 110 (M+1), (Yield: 75%).

Procedure for the preparation of *N*-(3-hydroxyphenyl) acetamide (metacetamol) (3): Compound **(2)** (0.11 mol, 25 g) was dissolved in acetic anhydride (80 mL) and the reaction mixture was stirred at 60 ^{0}C for 8 h at room temperature under nitrogen atmosphere. The excess acetic anhydride was removed under reduced pressure, the residue was dissolved in MDC, washed with water. The organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated to obtain metacetomol **(3).** LCMS: 152 (M+1), **(**Yield: 82%).

General Procedure for the preparation of alkylated metacetamol (5a-m): Equimolar quantities of metacetamol **(3)** (0.001 mol, 0.5 g), different substituted benzyl bromide **(4a-m)** (0.001 mol)**,** K_2CO_3 (0.003 mol, 0.57 g), were stirred in dry acetonitrile (10 mL) under nitrogen at room temperature for 12 h. The reaction mixture was filtered and the filtrate was diluted with ethyl acetate, washed with water and brine. The organic phase was dried over Na_2SO_4 and evaporated under vacuum, residue was purified by column chromatography using petroleum ether/ethyl acetate as eluent (7:3) to get alkylated metacetamol derivatives **(5a-m)** in good yield. Physical data of all the final compounds are represented in **table 1.**

Table 1: Physical data of final molecules **5a-m**

Compound	MP (^{0}C)	Yield (%)	Structure	LCMS (M+1)	Cal. (found) %		
					C	H	N
5a	142	55		276.2	65.34 (65.33)	5,12 (5.11)	5.08 (5.07)
5b	128	62		311.17	58.08 (58.07)	4.22 (4.21)	4.52 (4.52)
5c	157	61		256.2	75.27 (75.28)	6.71 (6.72)	5.49 (5.50)
5d	192	76		276.2	65.34 (65.34)	5.12 (5.11)	5.08 (5.06)
5e	158	52		256.2	75.27 (75.26)	6.71 (6.68)	5.49 (5.46)
5f	151	68		310.1	62.13 (62.14)	4.56 (4.57)	4.53 (4.54)
5g	142	71		272.1	70.83 (70.83)	6.32 (6.32)	5.16 (5.15)
5h	126	58		278.1	64.98 (64.97)	4.73 4.74)	5.05 (5.05)
5i	113	57		276.0	65.34 (65.32)	5.12 (5.11)	5.08 (5.09)
5j	127	64		278.1	64.98 (64.95)	4.73 (4.74)	5.05 (5.02)

5k	143	43		260.1	69.49 (69.49)	5.44 (5.45)	5.40 (5.41)
5l	111	65		320.1	60.17 (60.15)	5.37 (5.36)	4.39 (4.32)
5m	162	68		260.1	69.49 (69.46)	5.44 (5.41)	5.40 (5.38)

RESULTS AND DISCUSSION

As a first step 3-Aminophenol **(2)** was prepared from 3-nitrophenol **(1)** in the presence of palladium carbon in hydrogen atmosphere. This compound **(2)** was treated with acetic anhydride to give metacetamol **(3)** which was further alkylated with different substituted benzyl bromides **(4a-m)** to yield alkylated metacetamol **(5a-m)** in good yield. The synthesized derivatives were characterized by IR, ^{1}H NMR and ^{13}C NMR spectroscopy, the spectral results are tabulated in **table 4**. The newly synthesized compounds were tested for their antimicrobial and anthelmintic activities.

Table 4: IR, ^{1}H NMR and ^{13}C NMR of the final compounds 5a-m

Comp	IR	^{1}H NMR	^{13}C NMR
5a	IR (KBr,cm-1) : 3308 (C-H band), 2932 (N-H stretching),1664 (C=O), 1595 (C=C).	^{1}H NMR ; $CDCl_3$ (ppm): 7.43 (d, 2H, Ar-H); 7.2 (s, 3H, Ar-H); 7.2 (t, 2H, Ar-H); 6.9 (d, 1H, Ar-H); 6.7 (d, 1H, Ar-H); 5.0 (s, 2H, CH_2); 2.17 (s, 3H, CH_3).	^{13}C NMR; $CDCl_3$ (ppm): 173.2, 170.4, 163.8, 161.3, 154.5, 130.7, 130.6, 126.3 (2C), 123.5, 119.5, 111.4, 107.4, 80.8, 28.2.
5b	IR (KBr,cm-1) : 3386 (C-H band), 3029 (N-H stretching),1660 (C=O), 1560 (C=C).	^{1}H NMR ; $CDCl_3$ (ppm): 7.38 (d, 3H, Ar-H); 7.26 (s, 4H, Ar-H); 6.79 (d, 1H, Ar-H); 5.2 (s, 2H, Ar-H); 2.18(s,3H, CH_3).	----------
5c	IR (KBr,cm-1) : 3310 (C-H band), 3023 (N-H stretching),1665 (C=O), 1593 (C=C).	^{1}H NMR ; $CDCl_3$ (ppm): 7.34 (s, 1H, N-H); 7.2-7.12 (m, 6H, Ar-H); 6.9 (d, 1H, Ar-H); 6.7 (d, 1H, Ar-H); 5.0 (s, 2H, CH_2), 2.3 (s, 3H, CH_3), 2.1 (s, 3H, CH_3).	----------

5d	IR (KBr,cm-1) : 3283(C-H band), 3035 (N-H stretching),1669 (C=O), 1597 (C=C).	^{1}H NMR ; $CDCl_3$ (ppm): 7.5(s, 1H, N-H); 7.5-7.2 (m, 6H, Ar-H); 7.0 (d, 1H, Ar-H); 6.7 (d, 1H, Ar-H); 5.1 (s, 2H, CH_2); 2.1 (s, 3H, CH_3).	----------
5e	IR (KBr,cm-1) : 3303 (C-H band), 3022 (N-H stretching),1610 (C=O), 1592 (C=C).	^{1}H NMR ; $CDCl_3$ (ppm): 7.4 (s, 1H, N-H); 7.3-7.2 (m, 6H, Ar-H); 7.0 (s, 1H, Ar-H); 6.7 (d, 1H, Ar-H); 5.0 (s, 2H, CH_2); 2.3 (s, 3H, CH_3); 2.1 (s, 3H, CH_3).	----------
5f	IR (KBr,cm-1) : 3259 (C-H band), 2871 (N-H stretching),1664 (C=O), 1608 (C=C), 1342-1140 (CF_3 Streach)	----------	^{13}C NMR; $CDCl_3$ (ppm): 173.4, 170.1, 167.7, 161.5, 158.3, 155.8, 138.5, 130.7, 126.1, 119.0, 117.7, 114.8, 106.1, 96.1, 64.4, 22.03.
5g	IR (KBr,cm-1) : 3306 (C-H band), 2965 (N-H stretching),1670 (C=O), 1603 (C=C).	^{1}H NMR ; $CDCl_3$ (ppm): 7.3 (s, 1H, N-H); 7.3-7.2 (m, 2H, Ar-H); 7.0-6.9 (m, 2H, Ar-H); 6.8-6.7 (m, 2H, Ar-H); 6.7 (d, 1H, Ar-H): 6.7 (d, 1H, Ar-H): 5.0 (s, 2H, CH_2); 2.1 (s, 3H, CH_3); 1.6 (s, 3H, CH_3).	^{13}C NMR; $CDCl_3$ (ppm): 173.3, 170.3, 168.8, 149.4, 137.1, 130.6, 137.1, 130.6, 130.3, 126.1, 125.4, 122.4, 121.6, 119.7, 119.0, 104.0, 71.58.
5h	IR (KBr,cm-1) : 3271 (C-H band), 2960 (N-H stretching),1660 (C=O), 1596 (C=C).	^{1}H NMR ; $CDCl_3$ (ppm): 8.1 (s, 1H, N-H); 7.4-7.1 (m, 5H, Ar-H); 6.9 (d, 1H, Ar-H); 6.7 (d, 1H, Ar-H); 5.0 (s, 2H, CH_2); 2.1 (s, 3H, CH_3).	^{13}C NMR; $CDCl_3$ (ppm): 173.1, 170.3, 169.6, 149.4, 147.9, 146.5, 130.6, 126.1, 124.8, 120.3, 119.0, 108.9, 101.0, 70.9, 22.9.
5i	IR (KBr,cm-1) : 3266 (C-H band), 2962 (N-H stretching),1662 (C=O), 1596 (C=C).	----------	----------

5j	IR (KBr,cm-1) : 3259 (C-H band), 2871 (N-H stretching),1664 (C=O), 1608 (C=C).	^{1}H NMR ; $CDCl_3$ (ppm): 7.52 (s, 1H, N-H); 7.43 (d, 1H, Ar-H); 7.39-7.25 (m, 4H, Ar-H); 7.02 (d, 1H, Ar-H); 6.73 (d, 1H, Ar-H): 5.12 (s, 2H, CH_2); 2.19 (s, 3H, CH_3).	----------
5k	IR (KBr,cm-1) : 3308 (C-H band), 2889 (N-H stretching),1666 (C=O), 1596 (C=C).	----------	----------
5l	IR (KBr,cm-1) : 3262 (C-H band), 2921 (N-H stretching),1662 (C=O), 1599 (C=C).	^{1}H NMR ; $CDCl_3$ (ppm): 7.97(s, 1H, N-H); 7.95-7.20 (m, 6H, Ar-H); 6.91 (d, 2H, Ar-H); 5.18 (s, 2H, CH_2); 3.07 (s, 3H, CH_3); 2.18 (s, 3H, CH_3).	----------
5m	IR (KBr,cm-1) : 3296 (C-H band), 2934 (N-H stretching),1667 (C=O), 1596 (C=C).	^{1}H NMR ; $CDCl_3$ (ppm): 7.52 (s, 1H, N-H); 7.34-7.18 (m, 6H, Ar-H); 6.76 (d, 2H, Ar-H); 6.75 (d, 2H, Ar-H); 5.15 (s, 2H, CH_2); 3.07 (s, 3H, CH_3); 2.2 (s, 3H, CH_3).	----------

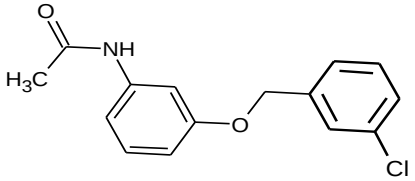
The IR spectrum of a representative compound **5a** shows C=O absorption and N-H vibration stretching in the range 1664 cm^{-1} and 2932 cm^{-1} respectively. Aromatic C-H band is displayed at 3308 cm^{-1}. A weak band at 1595 cm^{-1} represents C=C bond of the aromatic ring.

The ^{1}H NMR spectrum of a representative compound **5a** is discussed below.

Aromatic protons including N-H appears in the range of 7.43-7.21 ppm and CH_2 protons appears at 5.01 ppm as singlet. The methyl protons which is attached to the C=O appears at 2.17 ppm as singlet.

The ^{13}C NMR spectrum of a representative compound **5a** is discussed below.

Carbonyl carbon appears at high shielded area at 173.2 ppm, ipso carbons appears in the range 170.4, 163.8, 161.3 and 154.5 ppm. Remaining aromatic carbons appear in the range 130.7 to 107.4 ppm and aliphatic carbons appear in the range 80.8 and 28.2 ppm. All these observations confirm the assigned structure for the compound.



5a

Antibacterial activity: The synthesized compounds **5a-m** were screened for antibacterial activity. Concentrations of test compounds 400 µg/µL were prepared using DMSO and were tested against *S. Aureus, S. Citreus, B. Polymyx and B. Cereus* bacterial stains by disc diffusion method [17-19] using ciprofloxacin as standard (5 µg/50 µL). The discs with 6.0 mm in diameter were prepared using filter paper. Discs were kept in screw capped bottle and sterilized at 140 ^{o}C for 1 h. Discs for the experiment were prepared by taking twice the amount of test compounds solution required for each disc and added to the bottle containing discs. Discs with different concentration of test compound were placed on the nutrient agar media in two sets on fresh bacteria seeded on agar media and incubated at 35 ^{o}C for 12 h. The minimum inhibitory concentration (MIC) was noted by observing the lowest concentration of the drug which resulted in inhibition of bacterial growth. Out of all the synthesized compounds, some of the compounds showed good antibacterial activities. All the values are tabulated in **table 2**.

Table 2: Antibacterial activities of metacetamol derivatives **5a-m**

Compound	***S.Aureus***	***S.Citreus***	***B. Polymyxa***	***B. cereus***
5a	00	18	19	18
5b	00	22	23	23
5c	00	06	04	09
5d	00	19	16	19
5e	02	05	04	02
5f	00	24	23	21
5g	00	07	05	06
5h	08	25	24	24
5i	00	16	19	16
5j	00	26	24	23
5k	00	06	10	04
5l	00	18	18	16
5m	00	06	03	05
CIPX	28	27	24	24

CIPX = Ciprofloxacin is used as a positive control, and the zone of inhibition is expressed in mm.

Anthelmintic Activity: Anthelmintic activity of **5a-m** compounds was tested using *Pheretima posthuma* (Indian Earthworm). Worms were maintained under normal vermicomposting medium with adequate supply of nourishment and water for about three weeks. Adult earthworms of approximately 4 cm in length and 0.2 - 0.3 cm in width were chosen for experiment. Different concentrations 50 and 100 mg of samples were evaluated as per the standard method reported [20]. Five groups each with six earth worms were taken. Each P. posthuma was washed separately with normal saline before the initiation of experimental procedure and placed into a 20 mL of normal saline. Group I earthworms were placed in 20 mL saline in a clean Petri plate and Group II earthworms were placed in 20 mL saline containing standard drug Piperazine citrate (50 mg/mL). Similarly, Group III to XV earthworms were placed in a 20 mL saline containing 100 mg/mL of test samples. Observation was done keeping time taken for paralysis and the time taken for death as objective and was documented in minutes. Paralysis time was analyzed based on behaviour of the worms with no revival body state in normal saline medium. Death was concluded based on total loss of motility with faded body colour and the result are illustrated in **table 3.**

Table 3: Anthelmintic activity of 5a-m against Pheretima posthuma

Test Samples	Concentration (mg/ml)	Time taken for paralysis(min)	Time taken for death(min)
Control	_	142.33±0.49	167.17±0.87
Piperazine citrate	50	39.17±0.48**	57.00±0.58**
5a	100	65.00±1.59	86.50±0.76
5b	100	42.00±0.82**	51.17±0.60**
5c	100	68.50±0.76	88.67±0.71
5d	100	49.33±1.23	59.00±0.58
5e	100	63.17±0.48	74.50±0.89
5f	100	29.33±1.45**	39.17±0.70**
5g	100	57.50±1.23	72.50±1.26
5h	100	32.50±1.12**	45.17±1.11**
5i	100	53.50±1.38	89.33±1.56
5j	100	26.00±2.28**	38.50±0.41**
5k	100	56.00±2.28	68.50±0.41
5l	100	36.00±2.28**	44.50±0.41**
5m	100	66.00±2.28	78.50±0.41

Values are the mean±S.E.M of three earthworms. Symbols represent statistical significance *p<0.05, **p<0.01, ns: not significant as compared to control group.

APPLICATIONS

The synthesized metacetamol derivatives showed significant antibacterial and anthelmintic activities and are promising lead molecules for the development of new drugs. Several other metacetamol derivatives can be synthesized to evaluate their biological activity.

CONCLUSIONS

In the present research, some novel alkylated metacetamol compounds were synthesized and screened for their antimicrobial and anthelmintic activities. The compounds **5b, 5f, 5h** and **5j** showed good activity against the tested bacteria. The compounds **5b, 5f, 5h, 5j** and **5l** possessed significant anthelmintic activity which may be due to the presence of sulphur and halogens in substituted metacetamol ring system.

ACKNOWLEDGMENTS

The authors would like to thank Sapala Organics, Hyderabad, Andhrapradesh, India, Sree Siddaganga College of Pharmacy, Tumkur, Karnataka, India for spectral studies and, Department of Biotechnology, Jnana Bharathi Campus, Bangalore University, Bangalore, Karnataka, India for the biological experiment.

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