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N-BENZYL-AMINO FUMARAMATES: A NOVEL CLASS OF ANTIBACTERIAL AND ANTIOXIDANTS

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ABSTRACT

A series of novel N-benzylamino fumaramates derivatives (F1-F6) were obtained by the condensation of benzylamine with maleic anhydride in presence of thionyl chloride gives N-benzylamino fumaramic acid which reacts with various aliphatic & aromatic alcohol derivatives yields corresponding fumaramates. The formation of the N-benzylamino fumaramates was confirmed by IR, NMR, Mass spectra and Elemental analysis. All the synthesized compounds were screened for antibacterial and antioxidant activities using ciprofloxacin and ascorbic acid as a reference standard which shows a moderate to high potency against reference standards.

KEYWORDS: N-benzylamino fumaramates; Antibacterial; Antioxidants; DPPH; Ciprofloxacin.

INTRODUCTION

After the ‘fall of man’, fighting disease has become a normal part of his existence. This he does by the use of compounds derived from nature. One such class of compounds is called antibiotics. In 1889, Vuillemin, a French bacteriologist, suggested using the word “antibiosis”, meaning “against life,” to describe the group of drugs that had action against microorganisms.[3] Selman Waksman, An American microbiologist and the discoverer of streptomycin later changed this term to antibiotic in 19424. The term “antibiotic” as coined by Selman Waksman is used to describe any substance produced by a microorganism that is antagonistic to the growth of other microorganisms in high dilution (Low concentration).This definition excluded substances that kill bacteria but are not produced by microorganisms such as gastric juices and hydrogen peroxide.[5]

Antibiotics today, with advances in medicinal chemistry, are semisynthetic modifications of various natural com-pounds.[6] These include betalactam antibiotics, which includes the penicillin, the cephalosporin and the carbapenems. Some antibiotic compounds are still isolated from living organisms like the aminoglycosides, whereas other antibiotics like the sulfonamides, the quinolones and the oxazolidinones are produced solely by chemical synthesis.[7] This implies that synthesis of antibiotic compounds plays an important and vital role in the fight against disease-causing organisms.

In light of emerging resistance to antibiotic drugs, it has become imperative to synthesize new drugs with improved activity to combat various illnesses that have developed. Resistance to existing treatments. The problem of resistance to antibiotics on the part of the microorganism, the adverse side effects associated with antibiotics in current use and the difficulty in obtaining these antibiotics in large (Commercial) quantities from their natural sources implies that newer antibiotics have to be constantly sought for, to address these problems to give man the needed advantage in the ongoing battle between microbes and men. Synthesis of previously characterized antibiotics with structural modifications to imbue desirable qualities or remove undesirable ones pro-vides a way to assist man in this great battle. During studies on screening for antibiotics that showed activity against bacteria resistant to various known antibiotics, a new antibiotic with a broad antibacterial spectrum was isolated from the whole agar culture of Streptomyces strain NR-7GGI. This Streptomyces species was called streptomyces kurssanovii and the isolated antibiotic referred to as fumaramidmycin.[8]

EXPERIMENTAL

MATERIALS AND METHODS

Melting points (mp) were taken in open capillaries on Thomas Hoover melting point apparatus and are uncorrected. The IR spectra were recorded in film or in potassium bromide disks on a Perkin-Elmer 398 spectrometer. The 1H spectra were recorded on a DPX-500 MHz Bruker FT-NMR spectrometer. The chemical shifts were reported as parts per million (δ ppm) tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained on a JEOL-SX-102 instrument using fast atom bombardment (FAB positive). Elemental analysis was performed on a Perkin-Elmer 2400 C, H, N analyzer and values were within the acceptable limits of the calculated values. The progress of the reaction was monitored on readymade silica gel plates (Merck) using chloroform-methanol (9:1) as a solvent system.

Iodine was used as a developing agent. Spectral data (IR, NMR and mass spectra) confirmed the structures of the synthesized compounds and the purity of these compounds was ascertained by microanalysis. Elemental (C,H,N) analysis indicated that the calculated and observed values were within the acceptable limits (± 0.4%). All chemicals and reagents were obtained from Aldrich (USA), Lancaster (UK) or Spectrochem Pvt. Ltd (India) and were used without further purification.

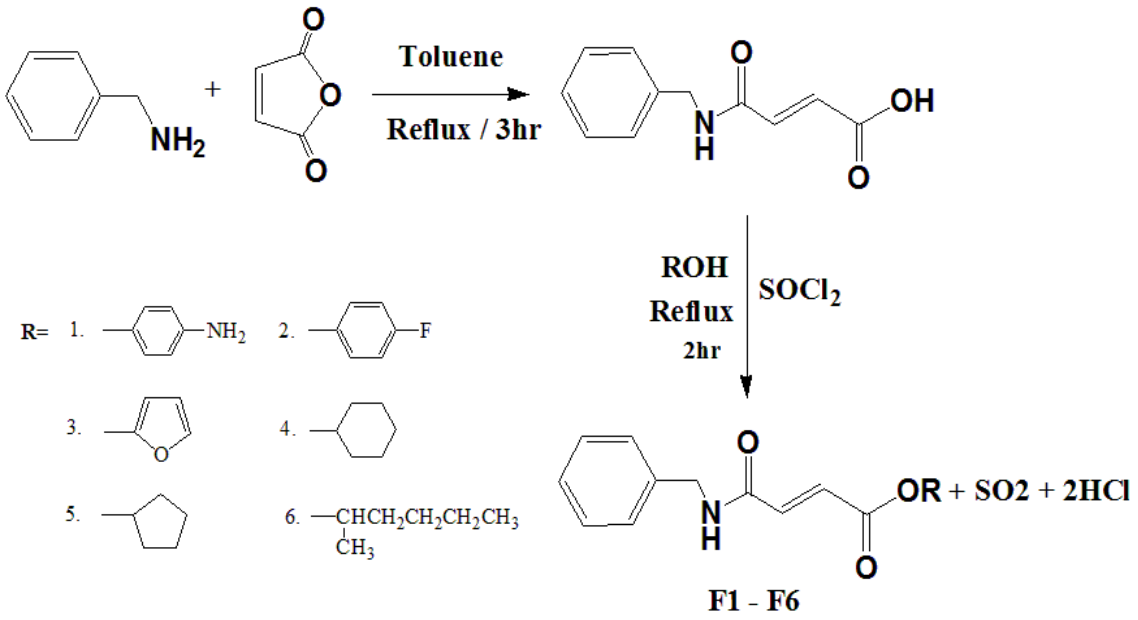
Chemistry

Synthesis of N-benzylamino fumaramic acid[21]: Benzylamine (1.0 g, 9.3 mmol) in toluene (5 mL) transfer into a round bottom flask containing maleic anhydride (1.1 g, 11.2 mmol) in toluene (5 mL). The mixture is to reflux with stirring for 3 hrs. The reaction is allowed to cool to room temperature and filter it using a Buchner funnel. The residue has to wash using ethyl acetate and dried to afford a shiny white solid. TLC is used to determine that the reaction had gone to completion.

Synthesis of N-benzylamino fumaramates[21]: This analogue is converted to its ester derivatives using resorcinol, propylene glycol, phenol, 2-hexanol, cyclohexanol and cyclopentanol respectively using a thionylchloride ($SOCl_2$) mediated esterification process according to reaction scheme 1.

Six portions of N-benzylamino fumaramic acid (0.5 g, 2.4 mmol) is individually transfer in to six round-bottomed flasks in ice baths. Thionyl chloride (2 mL) is to be added in drops with constant stirring. The excess thionyl chloride can be removed using a rotary evaporator and all the alcohols to be added to each flask and the reflux it. At the end of the reactions as determined by TLC, add saturated sodium carbonate (Na_2CO_3) solution to each flask until the solutions just turned alkaline as indicated by litmus paper. Add 20 ml of water to each flask and the mixtures are individually transferred to different separating funnels. The mixtures in the different separating funnels will be extracted extract using dichloromethane (2×25ml). Dry the combined dichloromethane fractions using anhydrous sodium sulphate (Na_2SO_4) and concentrate it to give clear oils. These are chromatographed on silica gel columns and eluted using benzene: ethyl acetate (6:4) to give the desired esters which crystallized on standing.

SCHEME



(E)-4-aminophenyl 4-(benzylamino)-4-oxobut-2-enoate (F1)

Yield 90%, mp 216–218 ºC; IR (KBr) cm^{-1}:3428 (NH), 3329 (NH_2), 3085 (Ar-CH), 2948 (C-H str), 1712, (C=O), 1611 (C=C str), 688 (C=C ben). ^1H NMR ($CDCl_3$) δ ppm; 4.10(s, 2H, NH_2), 4.22 (s, 2H, CH_2), 6.43 -6.82 (d, J = 8.0 Hz, 4H, Ar-H), 6.95 (s, J = 8.0Hz, 2H, Ar-H), 7.06-7.14 (m, J = 8.0Hz, 5H, Ar-H), 8.0 (s, 1H, NH). MS (m/z): 296 [M+]. Anal. Calcd for $C_{17}H_{16}N_2O_3$: C, 68.91; H, 5.44; N, 9.45.Found: C, 68.89; H, 5.44; N, 9.42.

(E)-4-fluorophenyl 4-(benzylamino)-4-oxobut-2-enoate (F2)

Yield 89.6%, mp 232-234ºC; IR (KBr) cm^{-1}: 3432 (NH), 3085 (Ar-CH), 2967 (C-H str), 1710 (C=O), 1606 (C=C str), 1428 & 538 (C-F). ^1H NMR ($CDCl_3$) δ ppm; 4.22 (s, 2H, CH2), 6.94 (s, J = 8.0Hz, 2H, Ar-H), 6.95 (s, J = 8.0Hz, 2H, CH), 7.05-7.07 (t, J = 8.0Hz, 5H, Ar-H), 7.14 (d, J = 8.0 Hz, 2H, Ar-H), 7.49 (s, J = 8.0 Hz, 1H, CH), 8.0 (s, 1H, NH) MS (m/z): 299[M+]; 301[M+2]. Anal. Calcd for $C_{17}H_{14}FNO_3$: C, 68.22; H, 4.71; N, 4.68 Found: C, 68.20; H, 4.71; N, 4.67.

(E)-Furan-2-yl 4-(benzylamino)-4-oxobut-2-enoate (F3)

Yield 76%, mp 243-245ºC; IR (KBr) cm^{-1}: 3367 (NH), 3058 (Ar-CH), 2910 (C-H str), 1698 (C=O), 1610 (C=C str), 743 (C=C bending). ^1H NMR ($CDCl_3$) δ ppm; 4.22 (s, 2H, CH_2), 6.3 (s, J = 8.0Hz, 2H, CH-CH), 6.95 (s, J = 8.0Hz, 2H, CH), 7.06-7.07 (t, J = 8.0Hz, 5H, Ar-H), 7.4 (d, J = 8.0 Hz, 1H, CH), 7.49 (s, J = 8.0 Hz, 1H, CH), 8.2 (s, 1H, NH). MS (m/z):

271[M+]. Anal. Calcd for $C_{15}H_{13}NO_4$: C, 66.41; H, 4.83; N, 5.16. Found: C, 66.40; H, 4.83; N, 5.15.

(E)-Cyclohexyl 4-(benzylamino)-4-oxobut-2-enoate (F4)

Yield 79.2%, mp 242-244ºC; IR (KBr) cm^{-1}: 3508 (NH), 3042 (Ar-CH), 1724(C=O), 1005 (CO-O-C), 652 (C=C bending). ^1H NMR ($CDCl_3$) δ ppm; 1.39 -1.80 (m, 8H, $(CH_2)_4$), 4.22 (s, 2H, CH2), 6.95 (s, J = 8.0Hz, 2H, CH), 7.06-7.14 (t, J = 8.0Hz, 5H, Ar-H), 7.49 (s, J = 8.0 Hz, 1H, CH), 8.4 (s, 1H, NH). MS (m/z): 287[M+]. Anal. Calcd for $C_{17}H_{21}NO_3$: C, 71.06; H, 7.37; N, 4.87. Found: C, 71.04; H, 7.36; N, 4.86.

(E)-Cyclopentyl 4-(benzylamino)-4-oxobut-2-enoate (F5)

Yield 68.4%, mp 262-264ºC; IR (KBr) cm^{-1}: 3522 (NH), 3043 (Ar-CH), 1710(C=O), 1667 (C=C str), 1006 (CO-O-C), 662 (C=C bending). ^1H NMR ($CDCl_3$) δ ppm; 1.46 -1.88 (d, 8H, $(CH_2)_4$), 4.20 (s, 2H, CH_2), 6.94 (s, J = 8.0Hz, 2H, CH), 7.06-7.14 (t, J = 8.0Hz, 5H, Ar-H), 7.49 (d, J = 8.0 Hz, 1H, CH), 8.3 (s, 1H, NH).MS (m/z): 273[M+]. Anal. Calcd for $C_{16}H_{19}NO_3$: C, 70.31; H, 7.01; N, 5.12. Found: C, 70.29; H, 7.01; N, 5.11.

(E)-Hexan-2-yl 4-(benzylamino)-4-oxobut-2-enoate (F6)

Yield 80.4%, mp 256-258ºC; IR (KBr) cm^{-1}: 3545 (NH), 3046 (Ar-CH), 1706 (C=O), 1667 (C=C str), 1005 (CO-O-C), 669 (C=C bending). ^1H NMR ($CDCl_3$) δ ppm; 1.06 -1.53 (m, 8H, $(CH_2)_4$), 4.13 (s, 2H, CH2),4.21(s, H, CH), (6.92 (s, J = 8.0Hz, 2H, CH), 7.06-7.14 (t, J = 8.0Hz, 5H, Ar-H), 7.46 (d, J = 8.0 Hz, 1H, CH), 8.1 (s, 1H, NH). MS (m/z): 289[M+]. Anal. Calcd for $C_{17}H_{23}NO_3$: C, 70.56; H, 8.01; N, 4.84.Found: C, 70.54; H, 7.99; N, 4.82.

RESULTS AND DISCUSSION

Pharmacological investigation

Acute toxicity studies

Acute toxicity study was performed for all the synthesized compounds to ascertain safe dose by acute oral toxic class method of Organization of Economic Co-operation and Development (OECD) - 423 guidelines. All the compounds tested for acute toxicity studies were also observed for gross behavioral changes in mice, continuously for 5 h at 1 h interval after administration of the compounds. The dose ranges of 5 mg/kg, 50 mg/kg, 500 mg/kg and 1000 mg/kg were used to test toxicity on animals as per guidelines. There after the observations were recorded intermittently for 24 h and compared with that of control group. In the behavioral profile, the animals have been observed for changes in their awareness and

mood. During the study, no deaths were observed for 14 consecutive days. From the study it was observed that all tested compounds were found to be safe even at 1000 mg/kg oral dose.

In-vitro antibacterial activity

All the tested compounds displayed promising anti-bacterial activity indicating their ability to inhibit growth of gram positive & gram negative microorganisms.[22] From the results (Table1) it was found that the compound cyclohexyl 4-(benzylamino)-4-oxobutanoate **(F5)** and hexan-2-yl 4-(benzylamino)-4-oxobutanoate **(F6)** was found to exhibit less anti-bacterial activity than title compound **(F1-F6)** and reference standard ciprofloxacin. Within the title compounds synthesized, an amino, fluro substituted and cyclohexanol derivatives **(F1,F2&F4)** showed better activity than corresponding hydroxyfuran, cyclopentanol and hexanol substituents **(F3,F5&F6)** against staphylococcus aureus, E-coli, Streptococcus pneumonia & klesiella pneumonia. Among various substituents tested on N-benzylamino fumaramates, compound possessing amino, fluro substituted and cyclohexane derivatives **(F1, F2&F4)** exhibited significant anti-bacterial activity, which was found to be more than the rest of substituents tested on micro-organisms. Replacement of amino, fluro and cyclohexanol groups by hydroxyfuran, cyclopentanol and hexanol results in decreased in anti-bacterial activity. Among the test compounds, Compound 4-fluorophenyl 4-(benzylamino) -4-oxobutanoate **(F1)** and compound cyclopentyl 4-(benzylamino)-4-oxobutanoate **(F4)** was found to have more potent activity against klesiella pneumonia species and moderate antibacterial activity against other organisms. Hence, the compound **4-fluorophenyl 4-(benzylamino)-4-oxobutanoate** (**F2**) was found to be the most active anti-bacterial agent against all four microorganisms such as staphylococcus aureus, E-coli, Streptococcus pneumonia & klesiella pneumonia. But **(F2)** exhibited moderately more potent activity than the reference standard ciprofloxacin.

In-vitro antioxidant activity

All the tested compounds displayed promising *in-vitro* antioxidant activity & indicating their ability to measure scavenging ability of antioxidants towards the stable DPPH radical.[23] The free radical DPPH is purple in color in methanol. Antioxidant reduces DPPH to 2, 2-diphenyl-1-picryl hydrazine, which is yellow in color. From the results (Table 2) it was found that the compound cyclohexyl 4-(benzylamino)-4-oxobutanoate **(F5)** and hexan-2-yl 4-(benzylamino)-4-oxobutanoate **(F6)** was found to exhibit less antioxidant activity than title compound **(F1-F6)** and reference standard ascorbic acid. Within the title compounds

synthesized, an amino, fluro substituted and cyclohexanol derivatives **(F1,F2&F4)** showed better activity than corresponding hydroxyfuran, cyclopentanol and hexanol substituents **(F3,F5&F6)**. Among various substituents tested on N-benzylamino fumaramates, compound possessing amino, fluro substituted and cyclohexane derivatives **(F1, F2&F4)** exhibited significant antioxidant activity, which was found to be more than the rest of substituents. Replacement of amino, fluro and cyclohexanol groups by hydroxyfuran, cyclopentanol and hexanol results in decreased DPPH scavenging antioxidant activity. The IC50 value was obtained by plotting percentage inhibition on y axis and concentration on x axis. Ascorbic acid was used as a standard and above procedure was followed to compare the antioxidant activity of different synthesized compounds with ascorbic acid. Among the test compounds, compound **4-fluorophenyl 4-(benzylamino)-4-oxobutanoate** (**F2**) (percentage inhibition is (**86.87%)** was found to be the most active antioxidant agent.

Table 1: Antibacterial screening of the synthesized compounds.

S. no.	Diameter of zone of inhibition in mm			
	Staphylococcus Aureus	E. Coli.	Klebsiella Pneumoniae	Streptococcus Pneumoniae
1	8	8	7	9
2	9	9	9	9
3	6	9	7	9
4	9	9	7	7
5	7	6	6	6
6	7	6	6	7
STD	10	10	10	10

Table no. 2. Antioxidant activity of synthesized compounds by DPPH method.

S. no	Compound Name	% Inhibition	IC_{50}± SEM DPPH Method
1	F1	32.0 -82.12	34.96±1.633
2	F2	1.85 – 86.87	32.34± 1.731
3	F3	2.60 ± 80.42	72.13 ± 1.189
4	F4	3.42 ± 80.12	60.29 ± 1.286
5	F5	2.75 ± 81.07	43.76 ± 1.898
6	F6	6.85 ± 79.56	45.51 ± 1.536
7	STD(Ascorbic Acid)	44.9 -95.5	12.7 ± 0.68

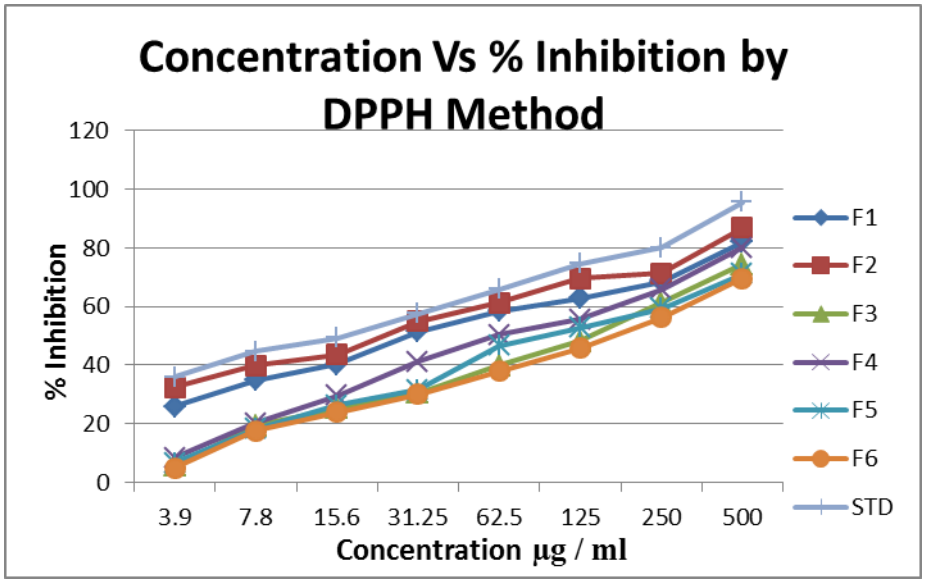


Fig. 1: Antioxidant screening of the synthesized compounds.

CONCLUSION

Among the test compounds, the compound 4-fluorophenyl 4-(benzylamino)-4-oxobutanoate **(F2)** was found to be most active anti-bacterial agent against staphylococcus aureus, E-coli, Streptococcus pneumonia & klesiella pneumonia which is moderately more potent than the reference standard ciprofloxacin. Among the test compounds, the compound 4-fluorophenyl 4-(benzylamino)-4-oxobutanoate **(F2)** was found to be most active antioxidant agent (percentage DPPH inhibition is (**86.87%**) which is moderately more potent than the reference standard ascorbic acid. Among the results of both the biological activities, all the compounds were shown good antibacterial activity than anti-oxidant activity. Hence this molecule can be selected as a lead molecule of the present study for further exploitation.

REFERENCE

1. S. M. Aronson, "The naming of antibiotics," Medicine and Health, 1997; 80(6): 180.
2. M. Radetsky, "The discovery of penicillin," The Pediatric Infectious Disease Journal, 1996; 15(9): 811–818,
3. S. A. Waksman, What is an antibiotic or an antibiotic substance. Mycologia, 1947; 39(5): 565–569.
4. F.Von Nussbaum, M. Brands, B. Hinzen, S. Weigand, and D. Häbich, "Antibacterial natural products in medicinal chemistry—exodus or revival?" Angewandte Chemie—International Edition, 2006; 45(31): 5072–5129.

5. H. B. Maruyama, Y. Suhara, J. Suzuki Watanabe et al., "A new antibiotic, fumaramidmycin. I. Production, biological properties and characterization of producer strain," Journal of Antibiotics, 1975; 28(9): 636–647.
6. T. Hasegawa, M. Asai, K. Haibara, T. Yamano, H. Iwasaki, and M. Yoneda, "A new antibiotic, C-9154," Journal of Antibiotics, 1975; 28(9): 713–716.
7. Olubunmi Bolarin-Akinwande, Elizabeth Bello and Isaac Bello1.Synthesis, Characterization and in-vitro biological activity profile of some derivatives of C-9154 antibiotic, FUW Trends in Science & Technology Journal, 2018; 3(2B): 702 – 707.
8. Takemori AE, Larson DL, Portoghese PS The irreversible narcotic antagonistic and reversible agonistic properties of the fumaramate methyl ester derivative of naltrexone, European Journal of Pharmacology, 1981; 70(4): 445-451.
9. Elizabeth Bello, Isaac Bell, Olubunmi Bolarin Akinwande. Synthesis, Characterization and in vitro biological activity profile of some derivatives of C-9154 Antibiotic, FUW Trends in Science & Technology Journal, 2018; 3(2B): 702-707.