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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME AMIDE DERIVATIVES

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ABSTRACT

*A series of amide derivatives has been synthesized through one-pot method by condensing appropriate 4-oxo-4-(4-substituted phenyl)butanoic acid moiety with isoniazid. The amides have been evaluated for their antimicrobial activity (Minimum Inhibitory Concentration - MIC) against Bacillus subtilis, Klebsiella pneumoniae and Candida albicans. One compound, **2b**, was found to have significant antimicrobial activity.*

RESUMO

Uma série de derivados de amidas foi sintetizada condensando o ácido 4-oxo-4-(4-fenil substituído) butanóico apropriado com isoniazida.. A atividade antimicrobial (Concentração Inibitória Mínima - CIM) foi avaliada com Bacillus subtilis, Klebsiella pneumoniae e Candida albicans. O composto 2b exibiu atividade antimicrobial significante.

INTRODUCTION

Over the past few decades the bacterial resistance to antibiotics has become one of the most important problems of infections treatment (1). Searching for new compounds, which would combine a non specific activity against a broad spectrum of bacteria and low toxicity seems to be a promising way to overcome that problem. Isoniazid is an important antibacterial drug and to increase its usefulness various derivatives have been synthesized with encouraging results (2).

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On the other hand, the derivatives of 4-oxo-4-(substituted phenyl)butanoic acid also show potential antimicrobial activities (3, 4). In view of these points and in continuation of our work on novel amides (1-3), it was considered worthwhile to study various amide derivatives of isoniazid with 4-oxo-4-(substituted phenyl)butanoic acids with a view to obtain potential antimicrobial agents. Therefore, five different 4-oxo-4-(substituted phenyl)butanoic acids were condensed with isoniazid and their structures were established on the basis of elemental analysis, ^{1}H NMR and Mass spectral data. These compounds were evaluated for their antimicrobial activities against some selected microbes.

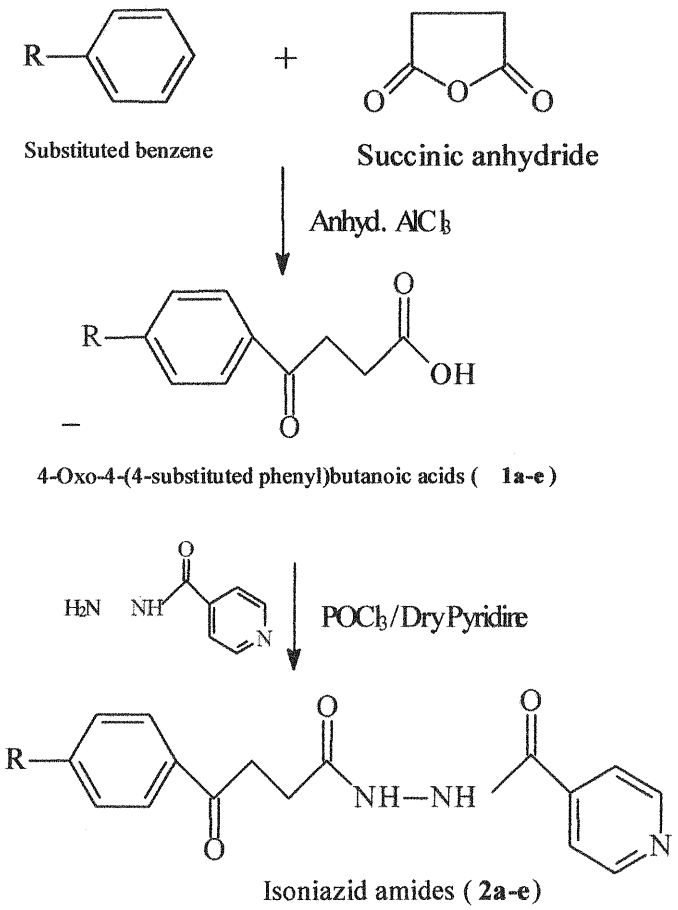
MATERIALS AND METHODS

Synthesis

Melting points were determined in open capillary tubes and are uncorrected. ^{1}H-NMR spectra were recorded on DPX-300 NMR spectrometer. The splitting pattern abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Mass spectra were recorded on a Jeol JMS-D 300 instrument fitted with a JMS 2000 data system at 70 eV. Microanalyses of the compounds were found within ±0.4% of the theoretical values. All solvents were distilled prior use. The progress of the reactions was monitored on silica gel G plates using iodine vapors as visualizing agent.

Synthesis of 4-oxo-4-(substituted phenyl)butanoic acid (1a-e)

These compounds were synthesized by following the method reported in literature (3).



Scheme 1. Protocol for synthesis of isoniazid amides (**2a-e**).

General procedure for the synthesis of Isoniazid amides (2a-e)

Amides were synthesized by dissolving 4-oxo-4-(substituted phenyl)butanoic acid (**1a-e**) (0.001 mol) and isoniazid (0.001 mol) in minimum quantity of dry pyridine separately. The two solutions were then mixed together and stirred magnetically followed by the addition of phosphorous oxychloride (0.9ml) drop wise while maintaining the temperature below 5°. The contents were stirred for another half-hour and left overnight. The reaction mixture was then poured into ice cold water and a solid mass, which separated out, was filtered, washed, dried and crystallized from ethanol to give **2a-e** (See Table 1).

Antimicrobial activity

All the newly synthesized compounds were screened for their antibacterial activity against *Bacillus subtilis*, *Klebsiella pneumoniaa* and *Candida albicans* at a concentration of 100 µg/ml by turbidity method (5). Compounds inhibiting growth of one or more of the above microorganisms were further tested for minimum inhibitory concentration (*MIC*). Solvent (DMF) and growth controls were kept. Minimum inhibitory concentrations (*MICs*) were determined by broth dilution technique. The nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and controls were inoculated with approximately $5x10^5$ c.f.u. of actively dividing bacteria cells. The cultures were incubated for 24 h at 37°C and the growth was monitored visually and spectrophotometrically. Ciprofloxacin and griseofulvin were used as standard drugs for comparison. The lowest concentration (highest dilution) required to arrest the growth of microbes was regarded as *MIC*.

RESULTS AND DISCUSSION

Synthesis

The synthesis of the title compounds was performed in a one-pot reaction method and is presented in Scheme 1. In the initial step, 4-oxo-4-(substituted phenyl)butanoic acid (**1a-e**) were prepared by condensing substituted benzenes with succinic anhydride in presence of anhydrous aluminium chloride following Friedel-Craft's acylation reaction conditions (3). The desired amides (**2a-e**) were synthesized by reacting 4-oxo-4-(substituted phenyl)butanoic acid (**1a-e**) with isoniazid in dry pyridine in presence of phosphorous oxychloride as condensing agent and obtained in appreciable yields (50-61%). The purity of the compounds was controlled by TLC in solvent system toluene:ethyl acetate:formic acid (5:4:1). Spectral data and microanalysis data were in agreement with the proposed structures. The physical and analytical data are recorded in **Table-1**.

The nuclear magnetic resonance spectra (^{1}H NMR; δ ppm) showed two triplets at around δ 2.8 & 3.3 (-CH_2-CH_2-); signals in the region δ 7.5-8.6 (aryl protons). The mass spectra showed molecular ion peaks in reasonable intensities supporting the structure. There was splitting of Ar-$COCH_2CH_2$-CON-bond resulting in formation of Ar-$COCH_2CH_2$-C≡O^+ (fragment-1) or [Ar-$COCH_2CH$=C=O]$^+$ (fragment-2) and/or C_5H_4N-C≡O^+. These fragments provided important clue for successful formation of the product. Fragment-1/2 further splitted to Ar-C≡O^+ and to Ar^+ and then to $C_6H_5^+$ (m/z=77).

Table 1. Physical and spectral data of the amide derivatives of isoniazid (**2a-e**).

Compd	R	M.p.; Yield (%)	Mol. formula; Mass spectral data (m/z)	^{1}H NMR spectral data (δ ppm)	Antimicrobial activity (*MIC*)		
					B. subtilis	*K. pneumoniae*	*C. albicans*
2a	H–	128-130 58	$C_{16}H_{15}N_3O_3$ 297 (M^+), 279, 160, 105, 78, 77	2.77 & 3.43 (t, each, 2x -CH_2), 7.56 (m, 3H, H-3,4,5, phenyl ring), 7.89 (m, 2H, H-2,6, phenyl ring), 8.25 & 8.73 (d, each, A_2B_2, 4H, 4-pyridyl ring), 9.16 & 9.83 (s, each, 2x -NH-).	>100	>100	50.0
2b	Cl–	172-174 55	$C_{16}H_{14}ClN_3O_3$ 331 (M^+), 332 (M^++1), 193, 139, 111, 53	2.78 & 3.31 (t, each, 2x -CH_2-CH_2-), 7.43 & 7.67 (d, each, A_2B_2, 4H, *p*-chloro phenyl ring), 8.06 & 8.77 (d, each, A_2B_2, 4H, 4-pyridyl ring), 9.28 & 9.65 (s, each, 2x -NH-).	12.5	25.0	12.5
2c	CH_3–	140-142 50	$C_{17}H_{17}N_3O_3$ 311 (M^+), 119, 91, 78, 77	2.39 (s, 3H, –CH_3), 2.63 & 3.26 (t, each, 2x -CH_2-), 7.28 & 7.88 (d, each, A_2B_2, 4H, *p*-tolyl ring), 7.81 & 8.79 (d, each, A_2B_2, 4H, 4-pyridyl ring), 9.11 & 9.36 (s, each, 2x -NH-).	25.0	50.0	25.0
2d	C_2H_5–	156-158 56	$C_{18}H_{19}N_3O_3$ 325 (M^+), 307, 133, 105, 91, 77	1.25 (t, 3H, –CH_3), 2.68 (q, 2H, –CH_2–), 2.73 & 3.34 (t, each, 2x -CH_2), 7.29 & 7.83 (d, each, A_2B_2, 4H, *p*-ethyl phenyl ring), 7.81 & 8.71 (d, each, A_2B_2, 4H, 4-pyridyl ring), 9.08 & 9.19 (s, each, 2x -NH-).	>100	>100	>100
2e	CH_3O–	148-150 61	$C_{17}H_{17}N_3O_4$ 327 (M^+), 191, 189, 135, 78	3.82 (s, 3H, –OCH_3), 2.78 & 3.30 (t, each, 2x -CH_2-), 6.96 & 7.56 (d, each, A_2B_2, 4H, *p*-methoxy phenyl ring), 7.91 & 8.83 (d, each, 4H, A_2B_2, 4-pyridyl ring), 9.23 & 10.11 (s, each, 2x -NH-).	50.0	>100	25.0
Standard-1†					6.25	6.25	nt
Standard-2†					nt	nt	6.25
Control					-	-	-

nt= not tested; †Standard-1 = Ciprofloxacin, Standard-2 = Griseofulvin; *MIC* = minimum inhibitory concentration

The fragment $C_5H_4N\text{-}C{\equiv}O^+$ further splitted to $C_5H_4N^+$. The fragmentation pattern is presented in **Chart 1**.

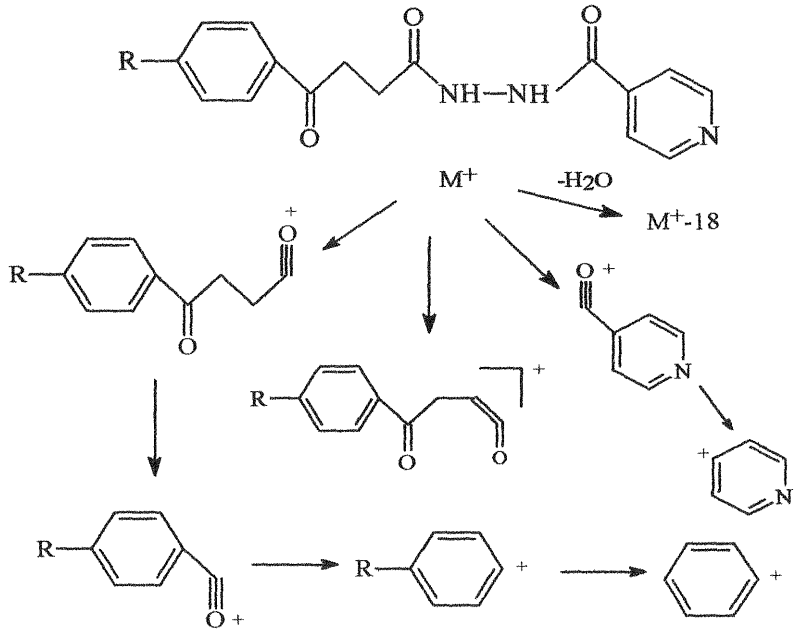


Chart 1. Proposed Mass fragmentation pattern of Amides

Antimicrobial activity

The antimicrobial activity (minimum inhibitory concentration - *MIC*) of the compounds was evaluated against *B. subtilis*, *K. pneumoniae* and *C. albicans*. Ciprofloxacin (*MIC*-6.25 μg/mL) and griseofulvin (*MIC*-6.25 μg/mL) were used as standard drugs for comparison. The compounds **2b** showed very good activity against *B. subtilis* and *C. albicans* with *MIC*-12.5 μg/mL and good activity against *K. pneumoniae* with *MIC*-25 μg/mL concentration. Another compound, compounds **2c**, showed significant activity against *B. subtilis* and *C. albicans* with *MIC*-25.0 μg/mL. Rest of the compounds were moderate in their action. From the antibacterial

results, it was observed that the compound having chloro function (**2b**) was most active among the tested compounds (**Table 1**).

CONCLUSION

In conclusion, five amide derivatives (**2a-e**) were successfully synthesized. Among these, one compound **2b** exhibited good activity against *B. subtilis* and *C. albicans* with *MIC*-12.5 μg/mL. These results showed the importance of exploring old drugs to obtain compounds of potential pharmaceutically interest.

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