

Synthesis of New Cyclic Amines-Linked Metronidazole Derivatives as Possible Prodrugs

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Abstract

Certain cyclic amine derivatives of metronidazole via acetate spacer were prepared. Cyclic amines used are piperidine and piperazine to improve the physicochemical properties and reduce some of metronidazole side effects. This is believed to be done by modification of its structural features to get prodrugs with improved properties over that of metronidazole. The present work includes esterification of metronidazole with chloroacetic acid, N-alkylation of the cyclic amines by the halogenated ester and characterization of their structures by spectral (UV and IR) and elemental (CHN) analysis. The melting points, degree of solubilities and partition coefficients were also determined. Both metronidazole-piperazine and metronidazole-piperidine derivatives have different structural and physicochemical properties that may be excellent to diminish problems associated with metronidazole.

Key words: Metronidazole, chloroacetate, prodrug.

الخلاصة

في هذا البحث نقوم بتحضير بعض المشتقات الامينية الحلقية للميترونيدازول من خلال جسر الخلات. ان الامينات الحلقية المستعملة هي البيرازين والبيريدين والغرض من هذا البحث هو تحسين الخواص الفيزيائية والكيميائية وتقليل بعض التأثيرات الجانبية للميترونيدازول. ان هذا يتم بأجراء تحويل في تركيبة الميترونيدازول للحصول على مقدمات ادوية ذات خصائص افضل من الميترونيدازول. يشمل الجانب العملي استرة الميترونيدازول مع الفا-كلورو حامض الخليك ثم تعويض المركب اعلاه على ذرة نتروجين الامينات الحلقية ومن ثم تشخيص هذه المركبات بواسطة التحليلات الطيفية (تحت الحمراء وفوق البنفسجية) وكذلك التحليل الدقيق للعناصر المكونة لها (كاربون-هيدروجين-نتروجين). كذلك فان درجات الانصهار، قابليات الذوبان والتوزع بين سائلين غير ممتازين قد تم تحديدها ايضا وكلها اثبتت حصولنا على مشتقين للميترونيدازول يمتلكان خواص فيزيائية وكيميائية وتركيبية تختلف عن الميترونيدازول وقد تكون افضل من حيث تقليل الجوانب السلبية له.

Introduction

Metronidazole is one of the nitroimidazole derivatives that are mostly used in treating many infections nowadays. It is effective against wide range bacterial, protozoal and parasitic infections^(1a). It has little more effect over mebendazole against *Giardia intestinalis* as well as intestinal nematodes⁽²⁾. Intravaginal metronidazole is effective in the treatment of bacterial vaginosis⁽³⁾. Metronidazole, as benzoyl form, could be used as supportive, suppressive and/or synergistic/additive drug in treatment of African trypanosomiasis⁽⁴⁾. Metronidazole can be considered as a prodrug in the sense that it requires metabolic activation by sensitive microorganisms in which the nitro group is reduced to hydroxylamine and covalently bind the DNA of the microorganism so that triggering the lethal effect^(1a,5). Metronidazole is rapidly and completely absorbed when given orally. It has a limited plasma protein binding, but can attain very favorable and rapid tissue distribution, including CNS and CSF⁽⁶⁾. Resistance to metronidazole can be

attributed to decreased activity of pyruvate: ferredoxin oxidoreductase which reduces metronidazole, induction of oxidative stress mechanism including superoxide dismutase and peroxiredoxin⁽⁷⁾, mutation of genes *rdxA*⁽⁸⁾ or lower activities of oxidases and reductases⁽⁹⁾. Increasing the dose and duration of treatment with acid suppression can overcome *H. pylori* resistance to metronidazole^(10,11). In therapeutic dose, the side effects of metronidazole include metallic bitter taste and CNS symptoms (dizziness, headache and sensory neuropathies). It also has a strong reported teratogenic effect⁽⁶⁾. In case of piperazine, it has the ability to dissolve uric acid, and so might be useful in gout and other rheumatic diseases. It is used as an effective anthelmintic in citrate form for the treatment of pinworms as well as round worms^(1b). Piperazine moiety was introduced to certain antibacterial drugs to increase activities of norfloxacin and ciprofloxacin against some bacteria^(1c).

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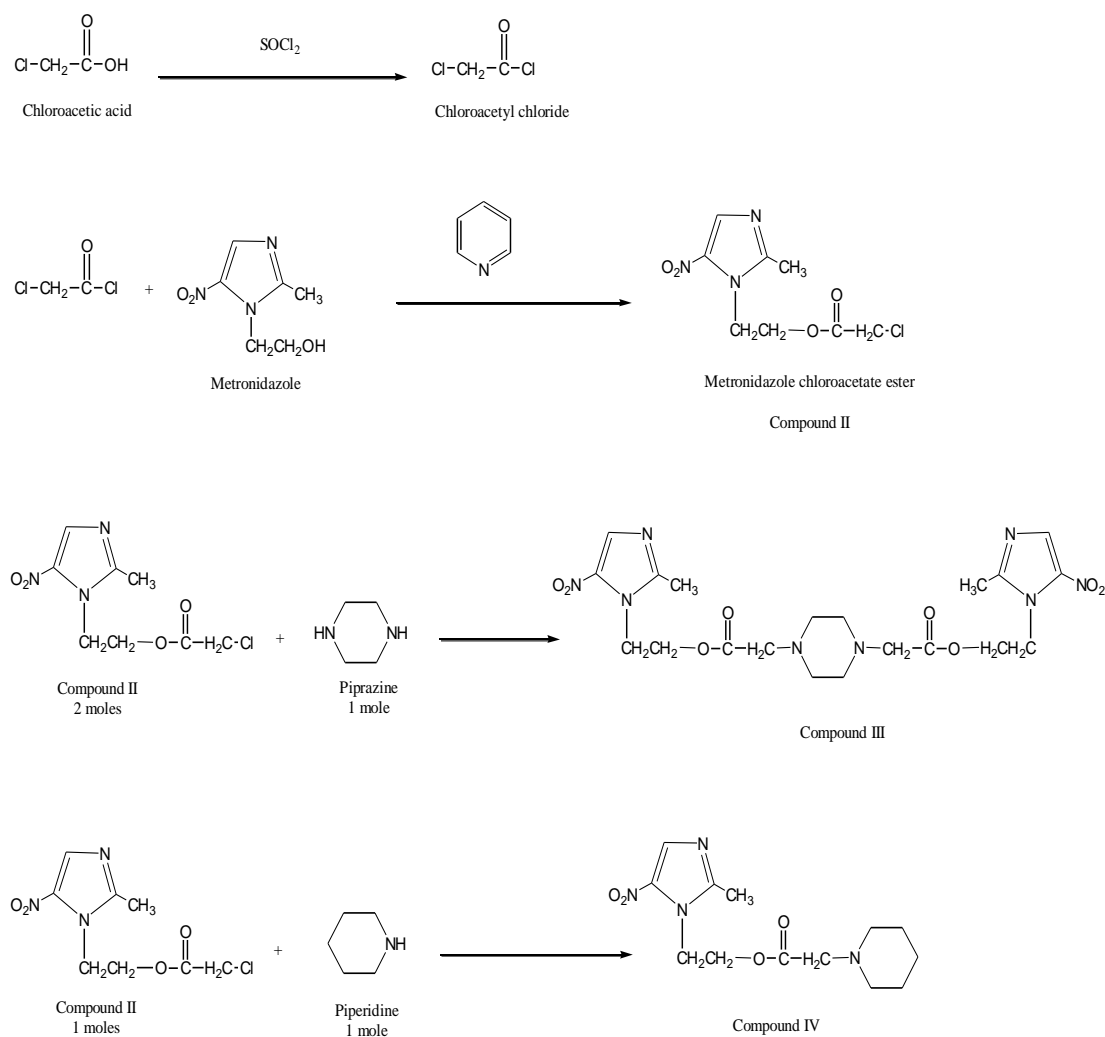
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It was found that parenteral combination of ciprofloxacin with metronidazole enhances the antimicrobial spectrum of quinolone derivatives against anaerobic organisms and gram-positive bacteria⁽¹²⁾. In cetirizine (H1-antihistaminic drug) the piperazine moiety participate in formation of zwitterions of the drug to prevent penetration into the brain and in turn minimizes CNS effects^(13,14). On the other hand piperidine is a cyclic amine and can be used as a moiety for the synthesis of many drugs such as the antihistamine, diphenylpyraline, which is structurally related to cetirizine^(15,14). Several of the functionalized piperidines have been shown to exhibit diverse biological activities through some enzyme inhibition such as glucosidase^(16,17), tryptase⁽¹⁸⁾ and some serine protease⁽¹⁹⁾. Both piperidine and piperazine moieties in fibrates structures were found to have superior activities in lowering serum triglycerides, cholesterol and sugar in mice and rats⁽²⁰⁾. Also many opioid receptors ligands are piperazine or piperidine-based agents⁽²¹⁾. Prodrugs of metronidazole; to reduce or abolish some of its side effects as well as improving its activities; like some amino acids^(22,23,24), organic acids⁽²⁵⁾ and others^(26,27,28) were synthesized. This field requires more investigations to get the most effective and suitable derivatives. Accordingly, we synthesized two derivatives of metronidazole using piperazine and piperidine linked via acetate bridging in order possibly to get a new suitable mutual ester prodrug with piperazine - diacetate or piperidine-acetate moiety; and these prodrugs (especially compound III) were supposed to be hydrolyzed to generate the parent drugs that may have gastrointestinal or systemic anthelmintic effects as in using diethyl carbamazine for ascaris and filareasis. This will occur in parallel to metronidazole release exhibiting its anti-protozoal and anti-anaerobic action; or a new derivative of possible better physicochemical and antibacterial properties since the presence of piperazine moiety might contribute in improving activity, as shown in ciprofloxacin antipseudomonal activity^(1c).

Materials and Methods

Metronidazole was supplied by SDI, Iraq; Piperazine hexahydrate and Methylene chloride (E. Merck AG, Germany); Piperidine, Chloroacetic acid and Pyridine (Fluka, Switzerland); Absolute ethanol 99% and

Thionyl chloride (BDH, England), Dioxan (Riedel-Dehean AG "Seelze-Hannover", Germany); Methanol and Chloroform (GCC, UK); while Cyclohexane by (Chem supply, South Australia). Methods of synthesis used were the conventional procedures (scheme 1)⁽²²⁾, melting points and TLC (R_f) values of intermediates and products were used to follow up the reactions, spectral analysis (UV and IR spectra) and elemental analysis (CHN analysis) to characterize the products were conducted. Partition coefficients and water solubilities were also determined. The synthesis started by activation of the carboxyl group of chloroacetic acid (15 gm, 158.73 mmole) with 15 ml thionyl chloride (SOCl_2) in 50 ml dried chloroform to produce chloroacetyl chloride, which (11.37 gm, 120 mmole) was reacted with metronidazole (15 gm, 87.66 mmole) in pyridine (7.95 gm, 100.63 mmole) and methylene chloride (125 ml) to give metronidazole chloroacetate ester as an intermediate (compound II). R_f values and CHN analysis were given in tables (2) and (3) respectively. Metronidazole - piperazine derivative (compound III) was prepared by reaction of compound II (9.9 gm, 40 mmole) dissolved in 125 ml dry dioxin with piperazine hexahydrate (1.942 gm, 10 mmole) dissolved in dry methanol. Compound III was then purified by washing with saturated sodium chloride solution, dried over anhydrous sodium sulphate and recrystallized from chloroform-cyclohexan mixture. The percent yield, physical description and melting point are given in table (1). R_f values and CHN analysis are given in tables (2) and (3) respectively. Metronidazole-piperidine (compound IV) was synthesized by the same method as for compound III except piperidine was used instead of piperazine solution and its properties were also shown in tables (1), (2) and (3). The partition coefficients of both compounds (III) and (IV) were determined by shaking 20 ml of 0.00012 M chloroform (organic solvent) solution of either compound with equal volume of distilled water for 30 min and their concentrations were measured spectrophotometrically at λ_{max} 310nm. A standard curve using series of concentrations of each compound in chloroform was constructed. The partition coefficients of the prepared compounds were calculated and shown in table (4)⁽²⁹⁾.



Scheme 1: Synthesis of compounds III and IV.

Table 1: Physical description, melting points and percent yield of metronidazole and compounds I, II, III and IV.

| Compound | Empirical formula | Molecular weight (gm/mole) | Description | % yield | M.P. OC (observed) |
|---------------|---|----------------------------|------------------------------------|---------|--------------------|
| Metronidazole | C ₆ H ₉ O ₃ N ₃ | 171.1 | Yellow crystals | --- | 159-161 |
| Compound I | C ₂ H ₂ OCl ₂ | 113.0 | Colorless-faint yellow oily liquid | 93 | --- |
| Compound II | C ₈ H ₁₀ O ₄ N ₃ Cl | 247.5 | Yellowish brown crystals | 76 | 70-72 |
| Compound III | C ₂₀ H ₂₈ O ₈ N ₈ | 508.5 | Off white powder | 61 | 201-203 (dec.) |
| Compound IV | C ₁₃ H ₂₀ O ₄ N ₄ | 296.0 | Deep yellow crystals | 78 | 106-109 |

Table2: R_f values of metronidazole, compound II, III and IV.

| Compound | R _f values | | |
|---------------|-----------------------|-------|-------|
| | A | B | C |
| Metronidazole | 0.662 | 0.851 | 0.611 |
| Compound II | 0.822 | 0.864 | 0.641 |
| Compound III | 0.39 | 0.54 | 0.269 |
| Compound IV | 0.794 | 0.826 | 0.723 |

Solvent system: A = chloroform : ethanol (4:1), B = chloroform : methanol (6:1), C = methanol alone.

Table 3: Elemental microanalysis of metronidazole and compounds II, III and IV.

| Compound | Molecular weight (g/mole) | Empirical formula | Element | Elemental analysis | |
|---------------|---------------------------|---|---------|--------------------|------------|
| | | | | % Calculated | % Observed |
| Metronidazole | 171.1 | C ₆ H ₉ O ₃ N ₃ | C | 42.10 | 41.96 |
| | | | H | 5.26 | 5.190 |
| | | | N | 24.56 | 24.43 |
| Compound II | 247.5 | C ₈ H ₁₀ O ₄ N ₃ Cl | C | 38.78 | 38.65 |
| | | | H | 4.040 | 3.980 |
| | | | N | 16.96 | 16.84 |
| Compound III | 508.5 | C ₂₀ H ₂₈ O ₈ N ₈ | C | 47.24 | 46.98 |
| | | | H | 5.510 | 5.330 |
| | | | N | 22.04 | 21.86 |
| Compound IV | 296.0 | C ₁₃ H ₂₀ O ₄ N ₄ | C | 52.70 | 52.51 |
| | | | H | 6.750 | 6.580 |
| | | | N | 18.91 | 18.63 |

Table 4: Partition coefficients and water solubilities of metronidazole and compounds III and IV.

| Compound | (P) | Log (P) | Water solubility (mg/ml) |
|---------------|-------|----------|--------------------------|
| Metronidazole | 0.677 | ~ -0.169 | 10 |
| Compound III | 98.26 | ~ 1.99 | 0.13 |
| Compound IV | 6.800 | ~ 0.832 | 0.59 |

Results and Discussion

The products obtained were analyzed by measurement of physical properties like, melting points, R_f values, partition coefficients, solubilities, as well as spectral methods, including UV and IR and were supported by significant analysis of the empirical formula of metronidazole, compounds II, III and IV as shown in tables 1, 2 and 3 respectively. The UV spectra shown in figures (1 and 2) showed that compounds II, III and IV differ from metronidazole in their chemical constitutions. The higher partition coefficient and lower water solubility of products III and IV indicate a higher lipophilicity and in turn might enhance the absorption through biological compartments, even the blood-brain barrier, since log (P) values occur within the optimum value of (0-3) ⁽³⁰⁾. With respect to ultraviolet spectra using equal molar concentrations of these compounds in chloroform show the same λ_{max} with just small variations within 5nm (λ_{max} 313-317 nm). Changing metronidazole to compounds II, III and IV has no effect on the extent of conjugation as shown in figures (1 and 2) ⁽³¹⁾. This follows the principle stating that, when two or more chromophoric groups are present in a molecule and they are separated by two or more single bond, the effect on spectrum is usually additive and there is little electronic interaction between isolated chromophoric groups ^(32,33).

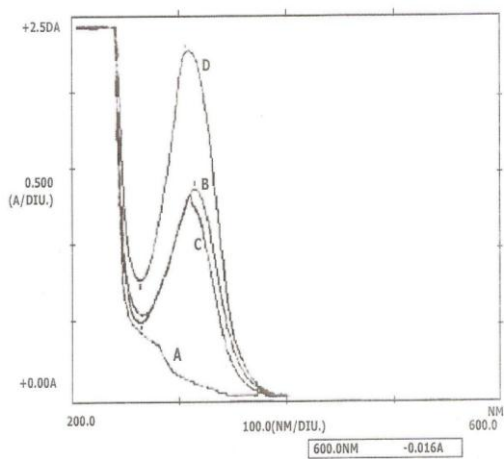


Figure 1: shows nearly equal λ_{max} (313 – 317) nm for Metronidazole (b),Compound II(C) and Compound III(D) using equal conc.(0.0008M) of the three compounds (A) for solvent .

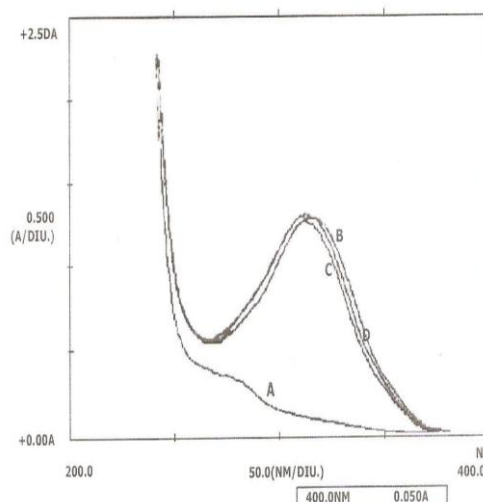


Figure 2 : shows nearly equal λ_{max} (313 – 317) nm for Metronidazole (b),Compound II(C) and Compound III(D) using equal conc.(0.00012M) of the three compounds (A) for solvent .

This is also seen in the extent of absorption intensity. The intensity of compound III (having molar absorptivity $\epsilon = 2.72 \times 10^4 \text{ mole}^{-1} \cdot \text{L} \cdot \text{cm}^{-1}$) is nearly twice that of metronidazole and compound II ($\epsilon = 1.61 \times 10^4 \text{ mole}^{-1} \cdot \text{L} \cdot \text{cm}^{-1}$), since compound III has double concentration of chromophore of metronidazole and compound II as shown in figure (1); whereas in figure (2), the intensities of absorption of metronidazole, compound II and IV ($\epsilon = 1.03 \times 10^4 \text{ mole}^{-1} \cdot \text{L} \cdot \text{cm}^{-1}$) are nearly equal, since they have the same concentration of chromophore.

The comparison of the IR spectra for piperazine, piperidine, metronidazole and compound II with those for compounds III and IV showed the following differences :

- Strong bands at (1715-1720) cm^{-1} and (1750) cm^{-1} due to ester (C=O) stretching vibration for compounds II , III and IV respectively.
- Two asymmetrical coupled bands at (1265 and 1180) cm^{-1} , (1245 and 1155) cm^{-1} and (1225 and 1193) cm^{-1} for (C-C(=O)-O) and (O-C-C) groups were appeared in compounds II, III and IV respectively.
- Two bands at (2870 and 2780) cm^{-1} due to asymmetrical and symmetrical stretching vibrations and two bands within fingerprint region at (1440 and 1380) cm^{-1} due to (C-H) bending vibrations of methyl group.
- The disappearance of a single weak-band in lower than (3550-3200) cm^{-1} region which is due to (N-H) stretching of secondary amine in spectrum of compounds III and IV.

- Disappearance of the strong-broad band (3550-3200) cm^{-1} ; which corresponds to hydroxyl (OH) stretching vibrations ; in compounds II, III and IV spectra.
- A strong broad band between(910-712) cm^{-1} and medium broad band between (820-710) cm^{-1} in piperazine and piperidine spectra respectively arisen from secondary amine (N-H) wagging. Compounds III and IV spectra have no this band.
- In the lowest frequency region of the spectra, which is sensitive region, there is a prominent variation in spectra of compounds III and IV from that of compound II which shows (C-C1) stretching (850-550) cm^{-1} , and the (CH₂) wagging band of (CH₂Cl) group at (1300-1150) cm^{-1} .

Conclusion

Cross-linking metronidazole with either piperazine or piperidine via an ester spacer was done resulting in new compounds with different and possibly better physicochemical and biological properties. With respect to compound III, it was prepared as a new mutual ester prodrug (and also of the twin ester type) that may liberate, upon hydrolysis, piperazine derivative as anthelmintic in addition to the anti-infective activity of metronidazole. The preparation of prodrug containing an imidazole moiety was intended, which probably show broader spectrum against worms, protozoa and anaerobic bacteria. Therefore, compound III can be used alone as a single potent mutual prodrug without the need for drug combination.

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