Synthesis of new heterocyclic derivatives bearing benzimidazole moieties and evaluation of their biological activity as bacterial flocculating agents

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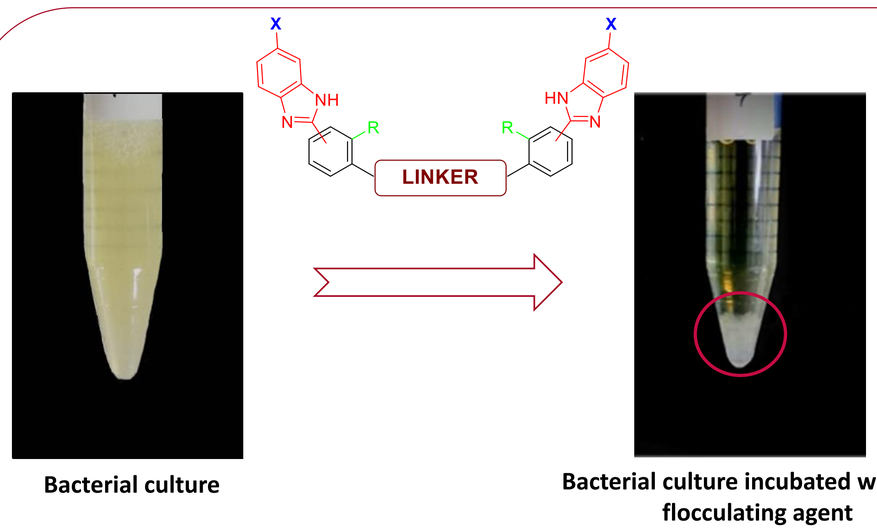
I. Introduction



Bacterial culture — **Bacterial culture incubated with a flocculating agent**

Figure 1: Phenomenon observed when incubating a bacterial culture with a bisbenzimidazole derivative

Bacterial flocculation is a biological process that has shown an increasing interest in recent years due to its various applications, ranging from wastewater treatment to biodegradation or biocatalytic processes. During this phenomenon, bacteria form eye-visible flocs. This process can be observed naturally but can also be enhanced using flocculating agents[(1)]. However, the flocculating agents currently developed exhibit various constraints preventing their use on an industrial scale. Among these, their mechanisms of action[(2)], their toxicity or their high cost may be a limiting factor.

As the benzimidazole moiety is found in many structures exhibiting interesting pharmacological activity (anti-viral, anti-fungal or even anti-bacterial activity[(3)]), we focused on the development of new heterocyclic derivatives bearing benzimidazole units. Interestingly, we have highlighted that some of these structures exhibit an important flocculation activity against both Gram (+) and Gram (-) bacterial strains. Moreover, we have demonstrated that these derivatives have a different flocculation mechanism than other existing systems.

II. Methods

The preparation of the targeted structures implies a two-step procedure.

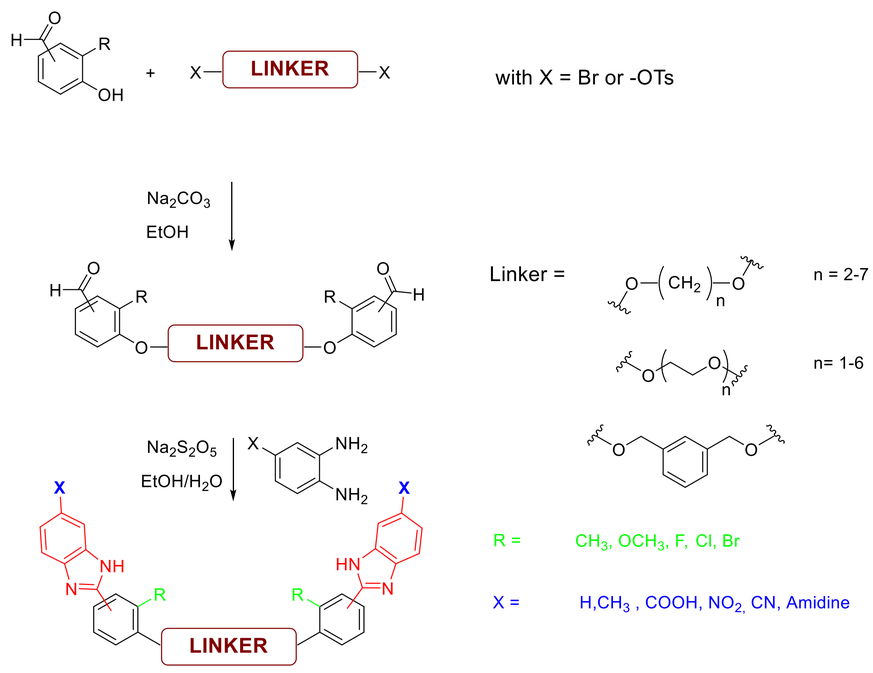


Figure 2: Reaction scheme used to obtain the bisbenzimidazole derivatives

All the obtained structures were characterized by ^{1}H NMR, infrared spectroscopy and mass spectrometry.

The biological activity of the new heterocyclic derivatives was then tested on two bacterial strains: *E.coli* (Gram (-) bacteria) and *L.rhamnosus* (Gram (+) bacteria).

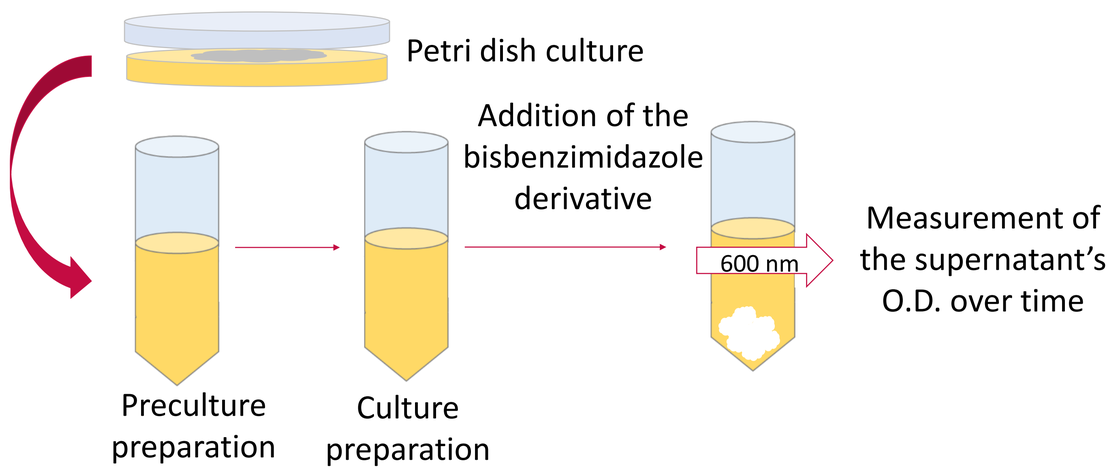


Figure 3: Microbial procedure used to evaluate the flocculation activity of the derivatives

The activity test is based on the incubation of a bacterial suspension with a low concentration of derivative. Over time, the optical density of the supernatant is measured, and the deposition of eye-visible flocs is observed.

Intrinsic study of the flocculation mechanism was also performed by using different approaches including microscopic studies (SEM, AFM, fluorescence microscopy,..) and other biological characterizations of the bacterial flocculate (metabonomics,..).

III. Results

Bacteriological tests

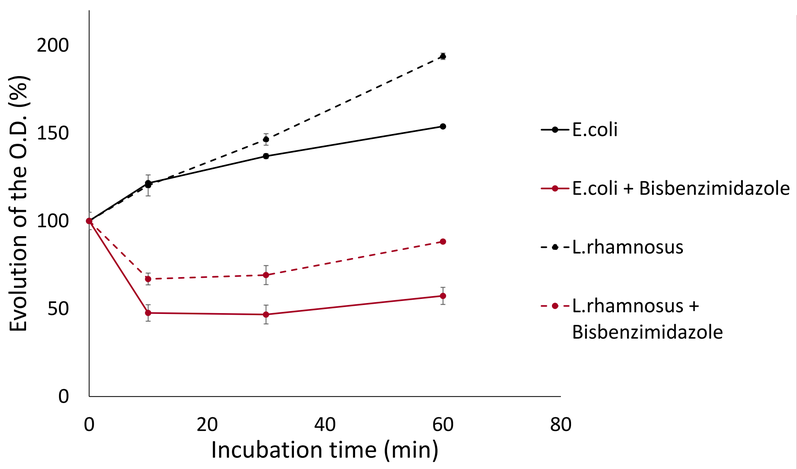


Figure 4: Evolution of the O.D. of a bacterial culture alone/incubated with a bisbenzimidazole derivative over time

- Decrease of the O.D over time when bacteria are incubated with a bisbenzimidazole compound;
- Efficient againt both Gram(+) (*Lactobacillus rhamnosus*) and Gram(-) (*Escherichia coli*) bacterial strains.

Microscopic studies

Scanning electron microscopy

No treatment — Bisbenzimidazole 30 min of incubation

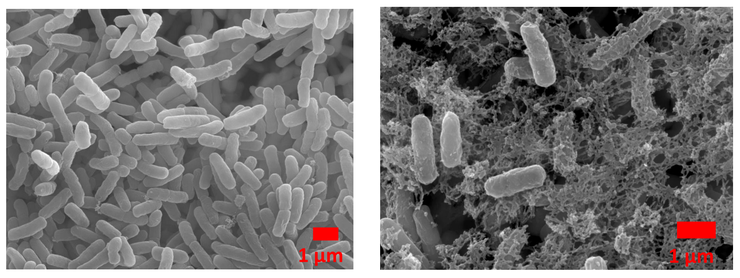


Figure 5: Bacterial culture alone/incubated with a bisbenzimidazole derivative

Atomic force microscopy

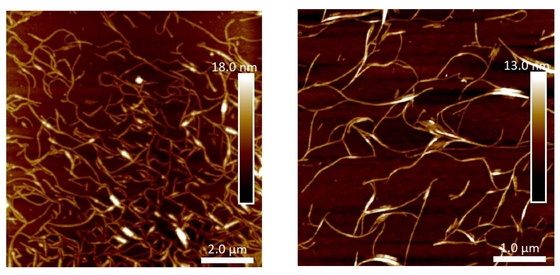


Figure 8: Self-assembled bisbenzimidazole molecules

Fluorescence microscopy

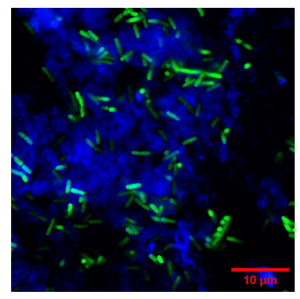


Figure 6: Bacterial flocculate induced by a bisbenzimidazole derivative (λ_{exc}: 318 nm, λ_{em}: 376 nm)

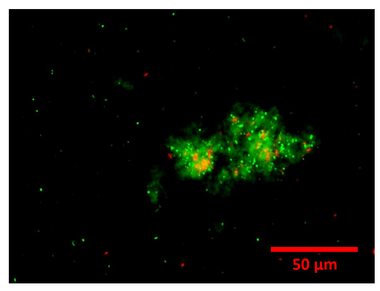


Figure 7: Live/Dead staining of a bacterial flocculate induced by a bisbenzimidazole derivative

Observations made through microscopic studies:

- After 30 min of incubation, appearance of a network entrapping bacterial cells;
- Localization of the derivative within the network, surrounding bacteria;
- Live/Dead staining: bacteria are mostly alive;
- Bisbenzimidazole self-assemble to form fibers in solution

➡ Self-assembly leads to bacterial flocculation

IV. Conclusion

Our study allowed to develop bisbenzimidazole derivatives that show an important bacterial flocculation activity against both Gram (+) and Gram (-) bacterial strains while providing an unusual mechanism of action. For those reasons, we believe that our derivatives offer an attractive prospect for the development of new agents with improved flocculation properties.

V. Acknowledgments

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References

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