# SYNTHESIS, ANTITUMOR ACTIVITY AND FORCED DEGRADATION OF ACYLHYDRAZONE BENZOXAZOLONE DERIVATIVES

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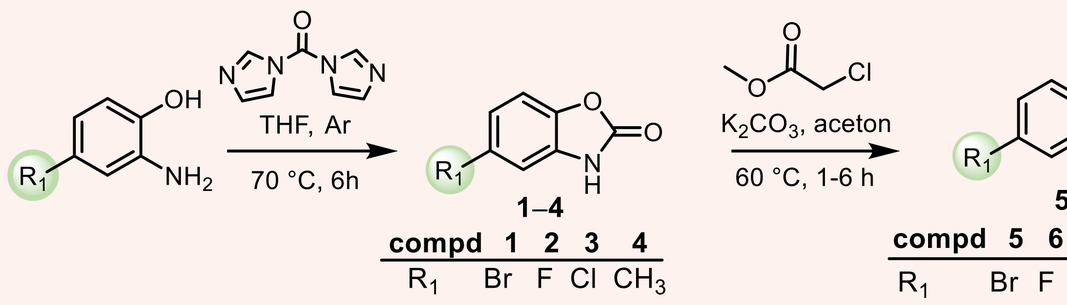
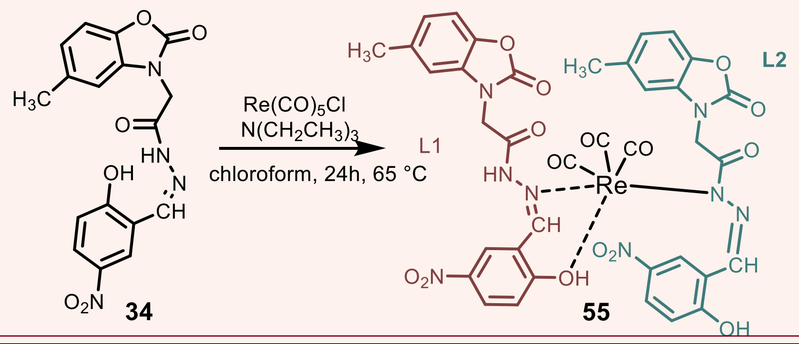
## BACKGROUND

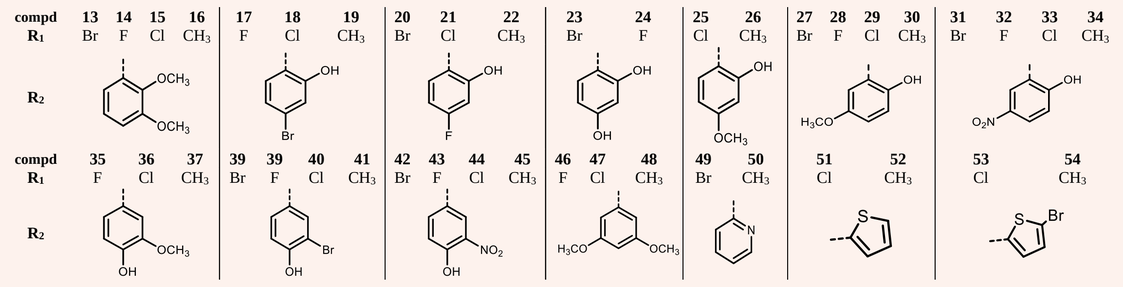
The benzo[*d*]oxazol-2-one core represents a promising scaffold in medicinal chemistry due to its favorable physicochemical properties and bioisosterism with purine DNA bases.[1] Furthermore, acylhydrazone derivatives exibit a wide range of biological activities including antitmor, antibacterial and antiviral effects, owing to the bioisosteric hydrazone subunit of the amide bond, making them promising candidates for modern drug design.[2]

## SYNTHESIS

The synthetic route involved cyclization of 4-substituted 2-aminophenols using 1,1′-carbonyldiimidazole to afford 5-substituted benzo[*d*]oxazol-2-ones **1**–**4**, which were then subjected to base-mediated alkylation with methyl chloroacetate to give the *N*-alkylated derivatives **5**–**8**, followed by reaction with hydrazine hydrate to afford the corresponding *N*-hydrazides **9**–**12**. The target acylhydrazone derivatives **13**–**54** were synthesized as *Z*-isomers *via* a mechanochemical approach involving the reaction of the benzoxazolone *N*-hydrazides **9**–**12** with various aromatic aldehydes using glacial acid. In addition, a tricarbonylrhenium(I) complex **55** of a selected acylhydrazone ligand **34** was successfully prepared with an addition of $Et_3N$.




## STRUCTURAL CHARACTERIZATION BY NMR

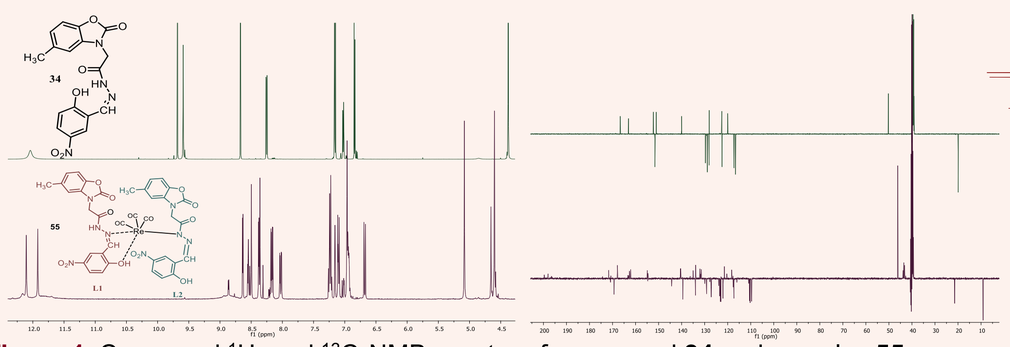


**Figure 1.** Compared $^1$H- and $^{13}$C-NMR spectra of compound **34** and complex **55**

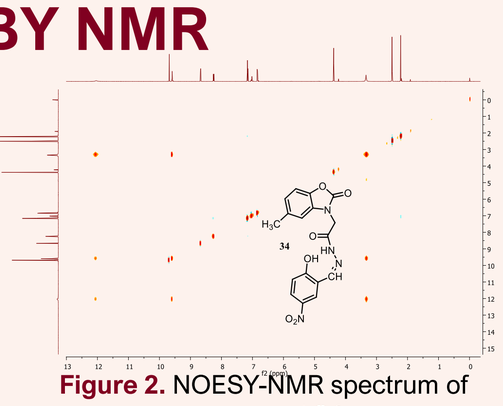


**Figure 2.** NOESY-NMR spectrum of compound **34**

## BIOLOGICAL EVALUATION

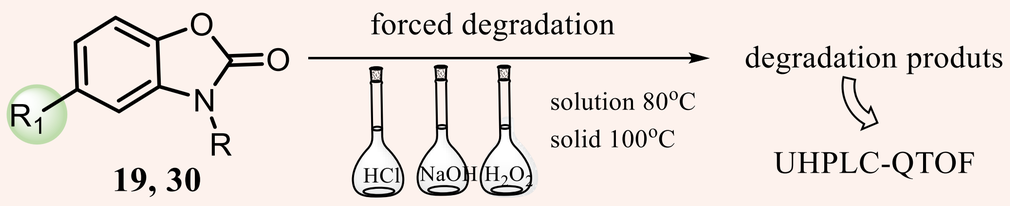
The *in vitro* antiproliferative activity of acylhydrazone benzoxazolone derivatives **13**–**54** was assesed against nine human tumor cell lines, with etoposide and nocodazole used as a reference compounds, and on normal cells PBMC to determine selectivity (Table 1).

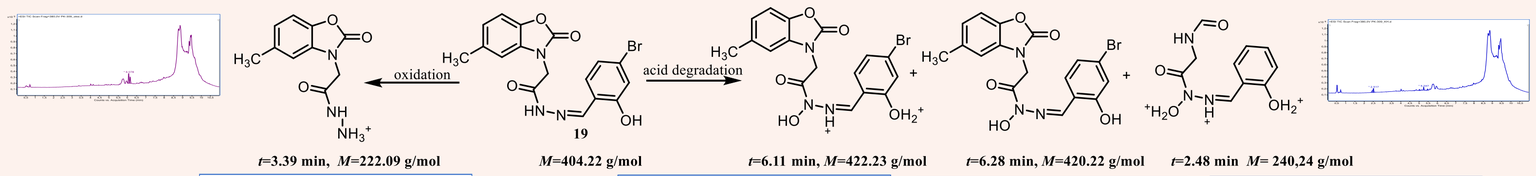
**Table 1.** *In vitro* antiproliferative activity of compounds **13**–**54**

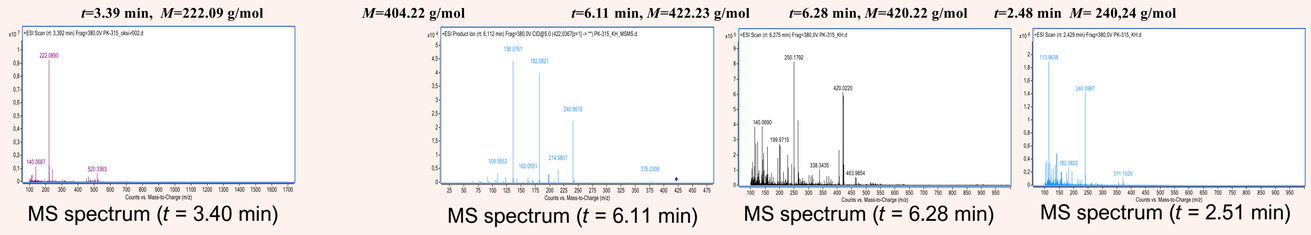
| | $IC_{50}$ / μM | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **COMPD** | **Capan-1** | **HCT-116** | **LN229** | **NCI-H460** | **SH-SY5Y** | **HL-60** | **K562** | **Z138** | **Molt-4** |
| **13** | >100 | >100 | 58.6 | >100 | n.d. | >100 | >100 | >100 | >100 |
| **14** | >100 | >100 | 83.4 | >100 | n.d. | >100 | >100 | >100 | >100 |
| **15–16** | >100 | >100 | >100 | >100 | n.d. | >100 | >100 | >100 | >100 |
| **17** | 3.4 | 7.3 | 37.3 | 5.9 | n.d. | 18.2 | >100 | 4.1 | **0.9** |
| **18** | 6.4 | 22.1 | 27.0 | 20.0 | 49.7 | 21.4 | 27.4 | 5.4 | 7.6 |
| **19** | **2.1** | 11.0 | 25.7 | 7.1 | 29.6 | 13.6 | 45.3 | 3.8 | 4.9 |
| **20** | 41.3 | >100 | >100 | >100 | n.d. | >100 | >100 | 91.5 | 27.8 |
| **21** | 5.4 | 10.5 | 99.9 | 89.4 | n.d. | >100 | >100 | 24.7 | 32.0 |
| **22** | 4.03 | >100 | >100 | >100 | 33.16 | 14.49 | 96.21 | >100 | 14.4 |
| **23** | 11.0 | 9.5 | 25.1 | 23.3 | n.d. | 54.5 | 36.7 | 12.2 | 4.5 |
| **24** | 68.2 | 31.2 | 81.1 | 26.2 | n.d. | >100 | >100 | 32.6 | >100 |
| **25** | 5.8 | >100 | >100 | >100 | >100 | >100 | >100 | 49.9 | 10.21 |
| **26** | 13.7 | >100 | >100 | >100 | >100 | >100 | >100 | 2.2 | 6.8 |
| **27** | 23.4 | 46.1 | >100 | 50.0 | n.d. | >100 | >100 | >100 | >100 |
| **28** | 16.4 | 20.4 | 28.3 | 17.6 | n.d. | 41.9 | 65.7 | 28.9 | 21.0 |
| **29–30** | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **31** | 15.6 | 26.7 | 62.6 | 47.9 | n.d. | 14.9 | 46.3 | 6.5 | 5.3 |
| **32** | 9.1 | 20.1 | 53.7 | 57.0 | n.d. | 13.1 | 47.8 | 14.2 | 5.6 |
| **33** | 5.6 | 33.7 | 74.5 | 51.8 | n.d. | 16.3 | 42.8 | 12.5 | 4.6 |
| **34** | 2.4 | 13.3 | 29.6 | 42.6 | 28.7 | 14.4 | 38.5 | 3.5 | 3.7 |
| **35–37** | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **38** | >100 | >100 | >100 | >100 | n.d. | >100 | 48.9 | >100 | >100 |
| **39–49** | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **50** | >100 | 40.6 | >100 | >100 | n.d. | >100 | >100 | >100 | >100 |
| **52–53** | >100 | >100 | >100 | >100 | n.d. | >100 | >100 | >100 | >100 |
| **54** | >100 | >100 | 70.72 | >100 | >100 | >100 | >100 | >100 | >100 |
| **Etoposide** | 0.3 | 1.3 | 5.8 | 1.2 | - | 0.5 | 5.2 | 0.6 | - |
| **Nocodazole** | 0.03 | 0.03 | 0.14 | 0.1 | - | 0.02 | 0.04 | 0.1 | - |

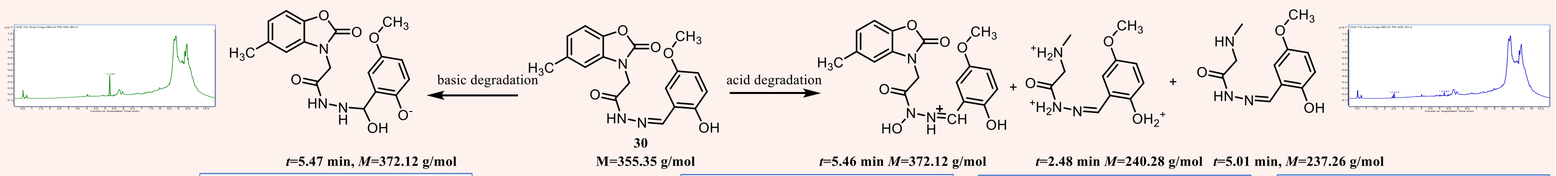
ADDITIONAL RESEARCH: Compounds **19, 34**: no DNA intercalating properties noted, no effect on microtubules noted, cell cycle arrest in G2/M phase, induction of DNA damage; $EC_{50}$ (PMBC) (**17–19**, **22**, **25**, **26**) = >50 μm, $EC_{50}$ (PMBC) (**34**) = 48.9 uM

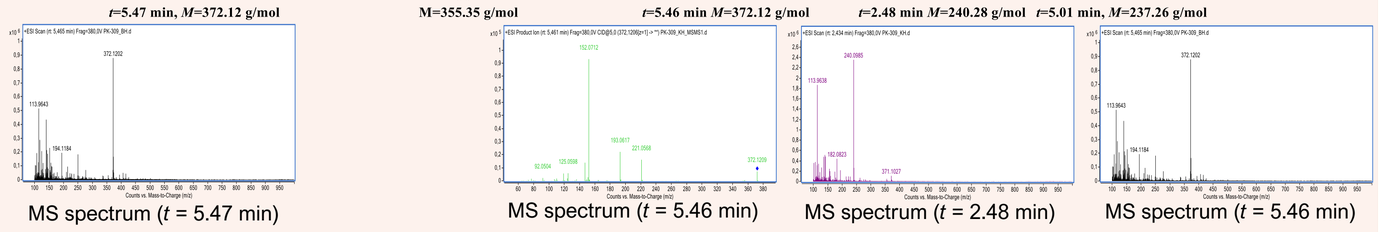
## FORCED DEGRADATION




## CONCLUSION

- Mechanochemical synthesis proved to be an efficient stereoselective method for preparing the *Z*-isomer of acylhydrazone derivatives of benzo[*d*]oxazol-2-one.
- The majority of the tested compounds exhibited ponunced to moderate antiproliferative activity against various human tumor cell lines; the most pronounced and selective antiproliferative activity was observed for compound **17** against the *T-cell acute lymphoblastic leukemia* cell line (Molt-4, $IC_{50}$ = 0.9 μM).
- Stability studies under stress conditions, including hydrolytic, thermal, and oxidative degradation, showed that compound **19** was susceptible to acid and oxidative conditions and compound **30** decayed under acid and base hydrolysis, with the resulting degradation products confirmed by mass spectrometry (MS).

## REFERENCES

[1] P. Prasher, T. Mall, M. Sharma, *Arch. Pharm*. 356 (2023) 42.
[2] L. Socea, S. Barbuceanu, E.M. Pahontu, A. Dumitru, G.M. Nitulescu, R.C. Sfetea, T. Apostol, *Molecules,* 27 (2022) 8719.

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